PB82-225764

Eye Irritation Testing: An Assessment of Methods and Guidelines for Testing Materials for Eye Irritancy

Tracor JITCO, Inc.
Rockville, MD

Prepared for

Environmental Protection Agency
Washington, DC

Dec 81

U.S. Department of Commerce
National Technical Information Service
NTIS

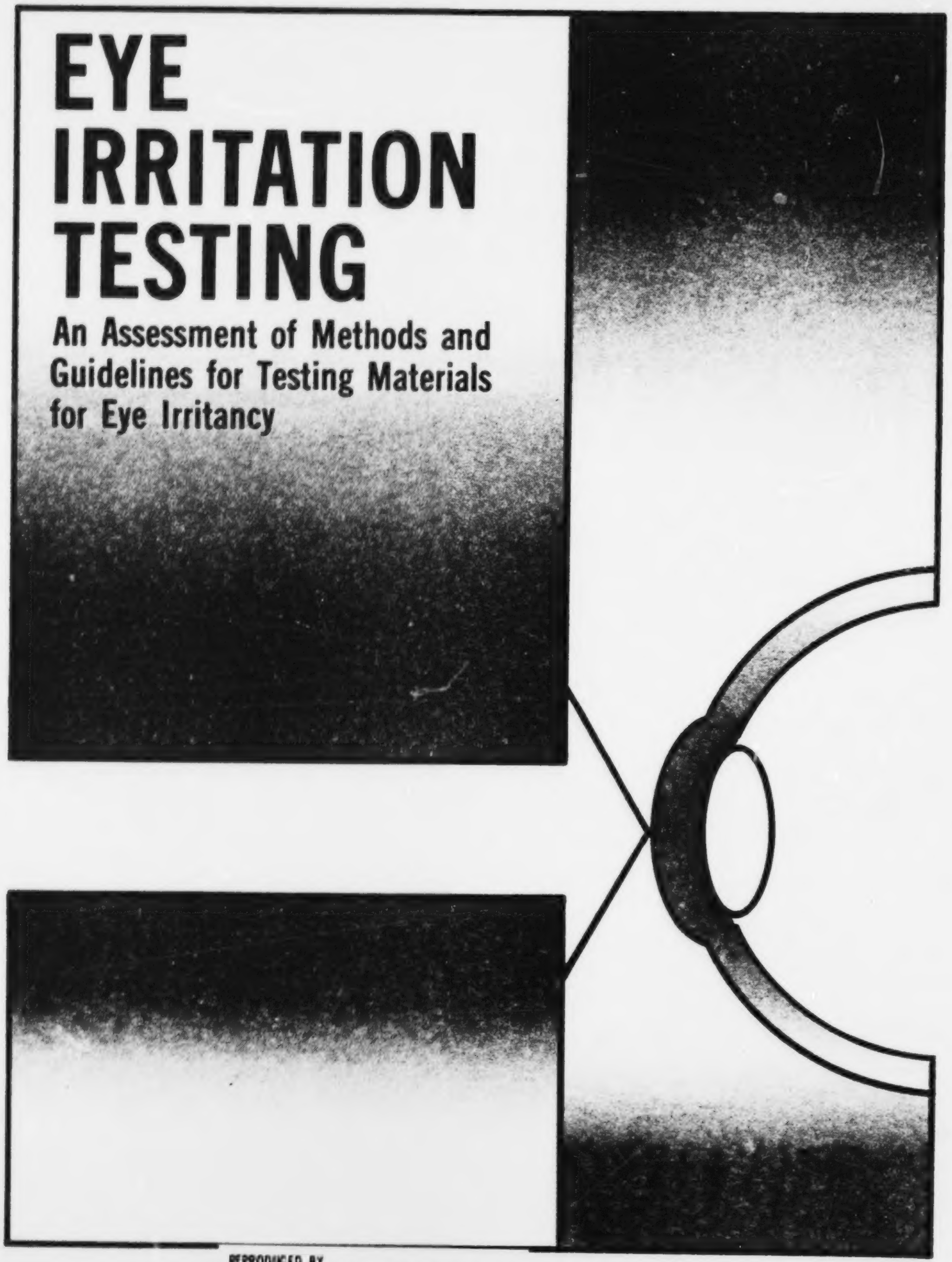
PB82-225764

EPA United States
Environmental Protection
Agency

Office of Pesticides and
Toxic Substances
Washington, D.C. 20460

EPA-560/11-82-001
October 1981

Pesticides and Toxic Substances



EYE IRRITATION TESTING

An Assessment of Methods and Guidelines for Testing Materials for Eye Irritancy

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

50272-101

REPORT DOCUMENTATION PAGE

1. REPORT NO
EPA 560/11-82-001

2.

3. Recipient's Accession No
PB82-225764

4. Title and Subtitle
EYE IRRITATION TESTING: An Assessment of Methods and Guidelines for Testing Materials for Eye Irritancy

5. Report Date
December 1981

6.

7. Author(s)
Falahee, K.J., Rose, C.S., Olin, S.S., Seifried, H.E.

8. Performing Organization Rept. No

9. Performing Organization Name and Address
Tracor Jitco, Inc.
1776 East Jefferson Street
Rockville, MD 20852-4081

10. Project/Task/Work Unit No
Work Assignment 02

11. Contract(C) or Grant(G) No
(C) 68-01-6176
(G)

12. Sponsoring Organization Name and Address
Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

13. Type of Report & Period Covered
Final Technical Report

14.

15. Supplementary Notes

16. Abstract (Limit 200 words)

The ocular safety of materials is determined primarily by observing the irritation produced by test agents instilled directly into the rabbit eye. The object of this study was to review the scientific basis for published guidelines, particularly those recently developed by the Organization for Economic Cooperation and Development (OECD) and the Interagency Regulatory Liaison Group (IRLG). These guidelines are essentially the same and recommend instillation of 0.1 ml material into the rabbit eye with observation for at least 72 hr. To increase cost-effectiveness and optimize use of animals, the guidelines reduce the number of animals and permit exclusions based on very high (≥ 11.5) or low (≤ 2) pH, and demonstrated dermal irritation. This is based on the high probability that agents meeting these criteria will be severe eye irritants. Data reviewed support excluding strong alkalies (pH ≥ 11.5). Exclusions of acids below pH 2 is not as well supported. Data indicate that the majority of severe dermal irritants would also be eye irritants. The monkey appears the most predictive animal model for human response; however, the rabbit still remains the species of choice due to practical considerations and the large data base which is useful for comparative purposes. Generally greater sensitivity of the rabbit eye compared to the human allows a conservative extrapolation to human risk. The development of alternative methodologies (e.g. *in vitro* tests) and the use of topical anesthetics is discussed. A tier strategy to eye irritation testing is proposed which screens substances on the basis of pH, dermal irritation and results from other tests.

17. Document Analysis a. Descriptors

Eye; Toxicology; Bioassay; Evaluation; Experimental Design; Toxic Tolerances; Laboratory Animals; In Vivo Analysis; In Vitro Analysis; Assessments

b. Identifiers/Open Ended Terms

Eye Irritation Testing Methods; Draize Method; Eye Irritants; Rabbits; Tier Testing; OECD Testing Guidelines; IRLG Testing Guidelines.

c. COSATI Field/Group

18. Availability Statement
Document is available to the public through the National Technical Information Service, Springfield, VA 22151

19. Security Class (This Report)

20. Security Class (This Page)

21. No. of Pages
100

22. Price

(See ANSI-Z39.18)

See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

EPA-560/11-82-001
October 1981

EYE IRRITATION TESTING

AN ASSESSMENT OF METHODS AND GUIDELINES FOR TESTING MATERIALS FOR EYE IRRITANCY

by

K.J. Falahee
C.S. Rose
S.S. Olin
H.E. Seifried

Contract No. 68-01-6176

Project Officer - Charles Auer
Technical Advisors - Norbert P. Page, Daljit Sawhney

Assessment Division
Office of Toxic Substances
Washington, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of its employees, contractors, subcontractors, or their employees, makes any warranty, expressed or implied, nor assumes any legal liability or responsibility for the accuracy or completeness of the information contained herein nor for any third party's use or the results of such use of any information, apparatus, product, or process disclosed in this report, nor represents that its use by such third party would not infringe privately owned rights.

This report has been reviewed by the Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the United States Government or the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation of use.

Preparation of this document was completed prior to the January 22, 1982 effective date of the EPA Administrator's Order 2200 and consequently did not necessarily undergo the peer review procedures described therein. The document received peer review according to procedures in place prior to that date and received complete administrative review.

Preceding page blank

TABLE OF CONTENTS

Page

PREFACE vii

EXECUTIVE SUMMARY ix

1.0 INTRODUCTION 1

2.0 HISTORICAL PERSPECTIVE 1

3.0 SURVEY OF TEST PROTOCOLS 5

3.1 TEST AGENTS 5
3.1.1 Corneal Penetration 6
3.1.2 pH 7
3.1.3 Results of Skin versus Eye Irritation 10

3.2 TEST AGENT VOLUME 11

3.3 METHOD OF EXPOSURE 12
3.3.1 Liquids and Solids 12
3.3.2 Aerosols 13
3.3.3 Corneal Applicators 14

3.4 DURATION OF EXPOSURE 14

3.5 SELECTION OF SPECIES 18
3.5.1 Use and Characteristics of Different Species 18
3.5.2 Comparison of Monkey and Rabbit Responses 20
3.5.3 Other Species 22

3.6 TEST GROUP SIZE 25

3.7 USE OF ANESTHETICS 26

3.8 OBSERVATION PERIOD 28

4.0 ASSESSMENT OF THE IRRITATION REACTION 29

4.1 EXAMINATION TECHNIQUES 29

4.2 SUBJECTIVE SCORING 30

4.3 OBJECTIVE METHODS OF ASSESSMENT 34
4.3.1 Corneal Thickness 34
4.3.2 Intraocular Pressure 36
4.3.3 Corneal and Conjunctival Edema 38
4.3.4 Capillary Permeability 38
4.3.5 Histological Investigation 39
4.3.6 Photographic Observation 40
4.3.7 Additional Techniques 40

Preceding page blank

TABLE OF CONTENTS (Continued)

Page

5.0 ADDITIONAL *IN VIVO* STUDIES 41
5.1 TESTS IN HUMANS 41
5.2 COMPLEMENTARY TESTS 43
6.0 *IN VITRO* TESTS 46
6.1 ISOLATED EYES AND CORNEAS 47
6.2 TISSUE CULTURE 49
7.0 EXTRAPOLATION OF ANIMAL DATA TO MAN 51
8.0 APPROACHES FOR THE FUTURE 55
9.0 CONCLUSIONS 56
10.0 RECOMMENDATIONS FOR FURTHER RESEARCH 62
11.0 REFERENCES 63

APPENDICES

Appendix A — Scale of Weighted Scores for Grading the Severity of Ocular Lesions 73
Appendix B — Test for Eye Irritants 75

TABLES

Table 1. Criticisms of the Draize/FHSA Test for Eye Irritation 2
Table 2. Ocular Irritation Test Methods 4
Table 3. Corneal Thickness of Several Species of Animals 19
Table 4. Comparison of Response of the Rabbit and Monkey Eye to Irritants 23
Table 5. Comparison of Response of Several Species to Eye Irritants 25
Table 6. Summary of Tissue Culture Tests for Assessing Eye Irritation 50
Table 7. Comparison of Animal and Human Responses 51

FIGURE

Figure 1. Tier Approach for Eye Irritation Testing 57

PREFACE

Recently, the Environmental Protection Agency (EPA) has been working with the Interagency Regulatory Liaison Group (IRLG) within the Federal government and with the international Organisation for Economic Cooperation and Development (OECD) to formulate a uniform set of toxicity testing guidelines. As part of this effort, EPA's Office of Pesticides and Toxic Substances requested Tracor Jitco to critically review, evaluate, and summarize the available information on eye irritation testing.

In recent years, there has been increased public attention and concern regarding the use of animals in scientific research. During the past year, a number of bills were introduced in the U.S. Congress related to the use of animals in research, including one to promote the development of methods of research, experimentation, and testing that minimize the use of live animals and another to establish a National Center for Alternative Research. Public and scientific concern in this area has been reflected in the formation of a number of special interest groups and in several recent symposia which considered alternative testing methods and procedures.

In the area of eye irritation testing specifically, much attention has been directed to the use of the rabbit. The Cosmetic, Toiletry, and Fragrance Association sponsored a workshop on ocular safety testing in October 1980 in which alternatives to the Draize rabbit eye test were a primary topic of discussion. In February 1981, the National Institutes of Health sponsored a symposium on Trends in Bioassay Methodology in which particular attention was focused on the feasibility of using *in vitro* and mathematical methods to reduce the dependency on the use of live animals in biological testing. Organizations represented at this symposium included academic institutions, industry, animal welfare groups, and government research and regulatory agencies. Progress in the development of *in vitro* and other alternative test methods is discussed in the present report.

In conducting this assessment, Tracor Jitco focused on the guidelines recently developed and promulgated by the OECD and the IRLG. On-line and manual searches of the relevant data bases were conducted and the significant literature was acquired and reviewed. Input on current developments was obtained through personal communication with investigators in the field and staff in several government agencies. The EPA provided the most recently developed versions of the OECD guidelines and contributed valuable background information concerning Federal and international efforts to standardize testing guidelines. The various protocols for eye irritation testing were examined and issues requiring further research based on these findings were identified.

The authors would like to thank Van Seabaugh of the Consumer Product Safety Commission for his advice during the preparation of this document and for providing the photographs contained in Appendix B of this report. Thanks are extended to Robert Osterberg of the Food and Drug Administration, James Murphy and Robert Jaeger of the EPA, and veterinary pathologist Robert Kovatch of Tracor Jitco for their review and helpful suggestions. The editorial assistance of Edward Cremmins, Marion Levy, and William Theriault (Tracor Jitco) is also gratefully acknowledged.

EXECUTIVE SUMMARY

A primary objective of toxicity testing guidelines is to ensure that the tests are scientifically sound, practical and cost effective, reliably predict effects in humans, and conform to acceptable standards for the care and use of laboratory animals. This report examines in detail the various protocols for eye irritation testing with particular reference to the guidelines published by the Interagency Regulatory Liaison Group (IRLG) and the Organisation for Economic Cooperation and Development (OECD). Characteristics of test agents which can influence the irritation response were considered along with the effect of test variables such as volume of test agent, method and duration of exposure, test group size, and species. Procedures for assessing the irritation reaction were considered in detail. Some complementary tests that have been developed to evaluate other responses of the eye to foreign substances (e.g., stinging) or that correlate with eye irritation also were reviewed. Finally, a comprehensive review was made of recent progress in the development of *in vitro* tests for eye irritation.

The historical antecedent for the eye irritation testing guidelines recently published by both the OECD and the IRLG is the Draize test, developed by Draize, Woodard, and Calvery in 1944. From 1964 to 1981, a modified Draize test has been specified by the Federal Hazardous Substances Act as the preferred method for eye irritation testing.

The eye irritation test as specified in the OECD and the IRLG guidelines utilizes a minimum of three albino rabbits. Substances are instilled into the conjunctival sac of the rabbit eye at a volume of 0.1 milliliter for liquids or 100 milligrams for solids. Aerosols are tested by a one-second spray directly into the eye from a distance of approximately 10 centimeters. Reactions are graded according to the severity of lesions produced in the cornea, iris, and conjunctiva at intervals throughout a 72-hour observation period (or longer when needed to evaluate the reversibility of a positive response).

In general, the specific experimental procedures recommended by the OECD and IRLG were found to be adequately supported by the experimental data evaluated in this review. Comments on the principal characteristics of the test methods follow.

The OECD and IRLG assumption that substances found to be severely irritating to the skin will also be severe eye irritants is valid for the vast majority of substances for which comparative data are available. Similarly, chemicals of pH 11.5 or greater or pH 2 or less generally are severe eye irritants, although the relationship at the acid end of the scale is less well-defined than at the alkaline end. Thus, the OECD suggestion that substances that are severe skin irritants or fall outside the pH 2-11.5 range need not be tested for eye irritation is supported.

Initial testing for eye irritation with three animals normally will be sufficient to identify substances that are non-irritating or maximally irritating. Testing with additional animals will be necessary to reliably characterize substances of intermediate degrees of irritancy.

An observation period of up to 72 hours, as specified in the guidelines, should be adequate for the qualitative identification of eye irritants. If a fuller characterization of the reversibility of persistent irritation or eye damage is desired, longer observation periods will be necessary.

The albino rabbit has been by far the species most commonly used in eye irritation testing since Draize and coworkers proposed their methods, and it remains the species of choice today. While the size and physical characteristics of the rabbit eye do simplify the test procedures, other factors are also important in the current preference for the rabbit. Where comparisons are possible, the rabbit eye appears to be at least as sensitive, and frequently more sensitive, to irritants than the human eye; thus, test results permit a conservative extrapolation in human risk assessment. In addition, evaluation of the significance of an irritant response can be placed on a firmer comparative basis with the rabbit, since eye irritation test scores are most meaningful when compared for a series of substances, and most test results available for comparison are from studies in rabbits.

The monkey eye is less sensitive than the rabbit eye to most substances and apparently is a somewhat more accurate predictor of human eye irritation response. The use of the rat for eye irritation testing has not been adequately studied. The OECD and IRLG guidelines both recognize the value of testing in species other than the rabbit for comparative purposes. However, limited information as well as practical considerations (particularly with monkeys) will continue to restrict this additional testing to those products for which a more in-depth evaluation is desired.

The direct instillation of 0.1 milliliter or 100 milligrams of the test substance into the conjunctival sac of the rabbit eye should be continued as the standard procedure, as recommended by OECD and IRLG, to allow correlation of results with the historical data base. However, the recent literature suggests that additional tests with smaller volumes or more dilute solutions can be especially valuable in ranking substances of similar irritancy and in correlating irritation responses in the test species with those resulting from typical accidental human exposures. Direct spraying of pressurized aerosols into the eye, while not readily comparable with the standard instillation procedure, has become the accepted method for tests of this type of product based on relevancy to human exposure.

Separate tests to determine the effect of flushing the exposed eye with water at a fixed interval after instillation of the test substance should remain optional, as provided for in the guidelines. Washing the eyes following exposure is generally beneficial in reducing the response to an irritant. The effects of this procedure are evaluated in this report.

Accuracy and reliability in the scoring of lesions have long been major goals in eye irritation testing and in the development of testing guidelines. The modified Draize scoring system is still considered adequate for grading irritant responses and the accuracy of scoring can be further enhanced by using a separate reference set of photographs of graded eye responses (see Appendix B). Interpretation of eye irritation scores for purposes of risk assessment requires a full evaluation of the data and their significance in potential human exposure situations.

Objective methods of measuring eye irritation can improve the uniformity and precision of the assessment process and enable the evaluation of more subtle effects and changes in the eye. Techniques that have been investigated include measurements of corneal thickness, intraocular pressure, capillary permeability, and corneal and conjunctival edema along with histological examination, photography, and specular and electron microscopy. These techniques should remain as optional means of supplementing conventional assessments.

Considerable efforts are being directed towards the improvement of the current animal test as well as the development of alternative methods. *In vitro* tests are being developed in an effort to create a rapid screening method and to reduce the use of animals in eye irritation testing. Tissue culture tests using corneal epithelial cell lines or other more common cell lines appear potentially beneficial as screening techniques and possible adjuncts or alternatives to the *in vivo* rabbit eye test. *In vitro* tests are limited, however, by their inability to demonstrate the whole animal response.

Several issues were identified from this review that could benefit from additional experimental research and development. These include:

- The continued development of *in vitro* tests
- Validation of an acceptable topical ocular anesthetic for use in the Draize test
- Better definition of the rabbit eye response to acids of pH less than 2
- Further investigation of test results using smaller volumes (or dilutions)
- Comparison of techniques for administering the test agent
- More extensive evaluation of other species (particularly rodents) for routine testing purposes.

A tiered approach to eye irritation testing is envisioned in which substances would be initially screened for pH, dermal irritation, and activity in *in vitro* eye irritation tests (when adequately validated). The need for testing in intact animals could thus be limited to substances of lower irritation potential or substances requiring a more complete characterization of the response. This approach should improve the eye irritation testing process in terms of cost-effectiveness and use of animal resources.

In summary, it is concluded from this review and evaluation of eye irritation testing methods that the 1981 OECD and IRLG guidelines are consistent with current practices and are adequately supported by the published work on this subject. The guidelines are by nature flexible and require the thoughtful and considered scientific input of the investigator in their application, interpretation, and evaluation. At the same time, there is sufficient standardization in the basic procedures to ensure general acceptability of the test results. The continuing development and validation of improved and alternative test methods is encouraged.

1.0 INTRODUCTION

Animal tests for assessing eye irritation and injury resulting from chemical exposure are designed to help predict the potential consequences of human ocular exposure to foreign materials. Data from such tests have been used to assess the degree of risk from numerous substances that present a potential exposure hazard during production, handling, distribution, and use. In recent years, significant improvements have been realized in the reliability, predictability, and reproducibility of eye irritation testing methods. Several reviews have been published during the past decade (Marzulli and Simon, 1971; Buehler, 1974; McDonald and Shadduck, 1977; Ballantyne and Swanston, 1977).

The Environmental Protection Agency (EPA) has been working in conjunction with the Interagency Regulatory Liaison Group (IRLG) and the international Organisation for Economic Cooperation and Development (OECD) to develop uniform guidelines for toxicity testing. Guidelines for eye irritation testing have recently been published in final form by both the IRLG and OECD. The present report, undertaken at the request of the EPA Office of Toxic Substances, reviews past and present test methodologies in relation to the guidelines. A number of aspects were singled out for consideration: regulatory criteria; methods and techniques; scoring and classification systems; species; significant modifications in existing methods; new methodology, both *in vivo* and *in vitro*; general predictability, sensitivity and accuracy of the tests; humane considerations; and comparisons of data from animals and humans.

The present review has utilized a broad base of information including the open literature, public comments submitted in response to proposed guidelines published by the EPA and the IRLG, and personal communication with investigators in government, academia, and industry. The information provides a basis for judging the adequacy and validity of present methods as well as a benchmark for comparison and evaluation of new or alternate methods and test systems.

2.0 HISTORICAL PERSPECTIVE

Draize, Woodard, and Calvery (1944) described a test in which albino rabbits were used to assess the potential of substances to cause eye irritation. This technique (Draize et al., 1944; Draize, 1959), commonly referred to as the Draize test, was derived from a method originally described by Friedenwald et al. (1944). The Draize test subjectively and quantitatively scores eye irritation by observing corneal, iridial, and conjunctival damage following instillation of a test material into the rabbit eye. One-tenth milliliter of the test material is placed in the conjunctival sac of one eye of the albino rabbit with the other eye serving as a control. A series of nine rabbits is used for each test. The treated eyes of three rabbits remain unwashed. The treated eyes of two other groups of three rabbits each are washed with 20 milliliters of lukewarm water approximately at body temperature two or four seconds after instillation of the test material. Ocular readings are made at 24, 48, and 72 hours and at 4 and 7 days after treatment or as long as injury persists. While the Draize test remains the basis of current eye irritation testing methods, many modifications of the original procedure have been developed and employed in the testing of chemical substances for experimental and regulatory purposes. Some aspects of the Draize test have also been questioned by various authors (Table 1).

Table 1. Criticisms of the Draize/FHSA Test for Eye Irritation

Humane considerations	Rowan (1980; 1981) Harriton (1981)
Discrepancies in response of the rabbit and human eye: test is performed in rabbits but results are applied to humans.	Buehler (1974) Marzulli and Simon (1971)
Method of exposure to test agent not comparable with means of human accidental exposure in the case of aerosols, powders, and granular substances.	Beckley (1965b)
Volume of materials as tested produces exaggerated results in the rabbit eye compared to findings in man.	Griffith et al. (1980)
Assessment of reaction is influenced by the test group size and the length of the observation period.	Weltman et al. (1965)
Subjective nature of the scoring system.	Buehler (1974) Heywood and James (1978)
Interlaboratory variability in tests with identical materials.	Russel and Hoch (1962) Rieger and Battista (1964) Weil and Scala (1971)
Difficulties in interpretation of test results.	Kay and Calandra (1962) Ballantyne and Swanston (1977)
Inability to correlate active inflammatory signs with permanent structural change.	Aronson (1975)

In the Federal Hazardous Substances Act (FHSA) (Federal Register, 1964) a modified Draize test was adopted as the official method of the Food and Drug Administration (FDA) for eye irritancy evaluation. A proposal for the modification of the FHSA method was put forth by the FDA in 1972 (Federal Register, 1972). This proposal, however, was never finalized and was eventually withdrawn officially in 1979 (Federal Register, 1979c).

In 1972, the responsibility for administering the FSHA was transferred to the Consumer Product Safety Commission (CPSC) upon the signing into law of the Consumer Product Safety Act (15 U.S.C. 2051; 1972). To assist in the evaluation of eye irritation test results, the CPSC in 1976 made available the "Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances" (CPSC, 1976), which was based on an earlier guide produced by the FDA (1965). Both guides are currently out of print. In addition, the FHSA method of ocular irritation testing has been published by the CPSC (Federal Register, 1979d). (The plates from the "Illustrated Guide" [CPSC, 1976] are included in Appendix B of the present report.)

At the request of the CPSC, the National Research Council of the National Academy of Sciences (NAS) reviewed the FHSA rabbit eye test and recommended a new protocol in the revision of NAS Publication 1138, "Principles and Procedures for Evaluating the Toxicity of Household Substances" (NAS, 1977). The objectives of this document were to revise and expand an earlier publication bearing the same title (NAS, 1964) in light of advancements in scientific technique, as well as to improve the accuracy and consistency of various toxicity testing methods including ocular irritation testing.

The Environmental Protection Agency proposed testing guidelines in 1978 for the development of ocular irritation data on pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Federal Register, 1978). Similar guidelines were proposed in 1979 for chemical substances and mixtures for which testing is required under the Toxic Substances Control Act (TSCA) (Federal Register, 1979a).

To resolve differences in regulatory policy among federal agencies requiring ocular irritation testing, the Interagency Regulatory Liaison Group proposed and published test guidelines in 1979 (Federal Register, 1979b). Public comments in response to the draft guidelines were considered in developing the final guidelines (IRLG, 1981).

The guidelines which have been developed and published by the Organisation for Economic Cooperation and Development (OECD, 1981) are similar to the published guidelines of the IRLG (1981). The IRLG and OECD guidelines will be periodically updated to implement changes in the "state-of-the-art." As indicated in a recent Federal Register notice (Federal Register, 1981a), the CPSC considers the test methods developed by the IRLG and OECD to be suitable for obtaining information on ocular irritation. Other test methods may be used with prior consent of the agency. While final standards have not yet been adopted by the EPA, the agency will consider proposed protocols which are consistent with the IRLG or OECD guidelines (Federal

Register, 1981a). On January 27, 1981, the EPA issued the following statement: ". . . the policy of the United States . . . is to pursue consistency in its test standards with the OECD Test Guidelines. They will have the same basic requirements so that data developed according to either EPA or OECD procedures with respect to those requirements should satisfy EPA needs."(Federal Register, 1981b). The FDA will use the IRLG guidelines as standard procedures for testing substances regulated under the Federal Food, Drug, and Cosmetic Act; but data derived using the OECD guidelines will also be acceptable. The FDA further recognizes that special tests may be required in some cases (Federal Register, 1981a).

Specific details of the above protocols and guidelines are presented in Table 2. The various testing criteria are discussed in the following section.

Table 2. Ocular Irritation Test Methods

	Draize et al. (1944) Draize (1959)	**FHSA (Federal Register, 1964; 1979d)**	**NAS (1977)**	**IRLG (1981)**	**OECD (1981)**
Test Species	Albino rabbit	Same	Same *(a)*	Same	Same
Age/Weight	NS *(b)*	NS	Sexually mature/less than 2 yr old	Young adult/2.0 to 3.0 kg	NS
Sex	NS	NS	Either sex	Either sex	NS
Number Animals/ Group	9	6	4 (minimum)	3 (preliminary test) *(c)*; 6	3 (minimum)
Test Agent: Volume and Method of Instillation					
Liquids	0.1 ml; direct instillation into lower conjunctival sac	Same as Draize	Liquids and solids: two or more different doses within the probable range of human exposure *(d)*	Same as Draize	Same as Draize
Solids	NS	100 mg or 0.1 ml equivalent when this volume weighs less than 100 mg; direct instillation into lower conjunctival sac	Manner of application should reflect probable route of accidental exposure	Same as FHSA	Same as FHSA
Aerosols *(e)*	NS	NS	Short burst at distance approximating self-induced eye exposure.	1 sec burst sprayed at approx. 4 inches	1 sec burst sprayed at 10 cm
Irrigation Schedule	At 2 sec (3 animals) and at 4 sec (3 animals) following instillation of test agent.	Eyes may be washed after 24 hr reading	May be conducted with separate experimental groups	Same as FHSA	Same as FHSA; in addition, for substances found to be irritating: wash at 4 sec (3 animals) and at 30 sec (3 animals)

Table 2. Ocular Irritation Test Methods (Continued)

	Draize et al. (1944) Draize (1959)	**FHSA (Federal Register, 1964; 1979d)**	**NAS (1977)**	**IRLG (1981)**	**OECD (1981)**
Irrigation Treatment	20 ml tap water (body temp.)	Sodium chloride solution U.S.P. or equivalent	NS	Same as FHSA	Wash with water for 5 min using volume and velocity of flow which will not cause injury
Examination Schedule	24 hr 48 hr 72 hr 4 days 7 days	24 hr 48 hr 72 hr	1 day 3 days 7 days 14 days 21 days	24 hr *(f)* 48 hr 72 hr	1 hr 24 hr 48 hr 72 hr
Use of Fluorescein	NS	May be applied after the 24 hr reading	May be used	Same as FHSA	Same as FHSA
Use of Anesthetics	NS	NS	NS	May be used	May be used
Scoring and Evaluation	Draize et al. (1944) *(g)*	CPSC (1976) *(h)*	Draize et al (1944) *(g)*, or a slit lamp scoring system	CPSC (1976) *(h)*	CPSC (1976) *(h)*

(a) Tests should be conducted in monkeys when confirmatory data are required.

(b) Not specified.

(c) If the substance produces corrosion, severe irritation, or no irritation in a preliminary test with 3 animals, no further testing is necessary. If equivocal responses occur, testing in at least 3 additional animals should be performed.

(d) Suggested doses are 0.1 and 0.05 ml for liquids.

(e) Currently no testing guidelines exist for gases or vapors.

(f) Eyes may also be examined at 1 hr, 7, 14, and 21 days.

(g) Presented in Appendix A.

(h) Presented in Appendix B.

3.0 SURVEY OF TEST PROTOCOLS

3.1 TEST AGENTS

The physical and chemical properties of a test substance can significantly influence the type and extent of damage produced in the eye. Occupational exposure to irritants can be in the form of liquids, solids, mists, or aerosols, vapors, and gases. For example, eye exposure data collected over a 12-month period from a chemical manufacturing plant showed that 52% of reported eye injuries caused by chemicals involved exposure to vapors and mists, 31% were caused by liquids, and 17% by solids (McLaughlin, 1946). The irritation produced by a test substance may be due to a combination of the physical form to which the eye is exposed (including particle size), and certain chemical and physical properties such as pH, lipid/water coefficient, and ionization. If the potential role of such properties in causing specific ocular reactions were established, a baseline for making testing decisions could be developed. For certain substances, this could entirely eliminate the need for testing. The OECD guidelines, for instance, state: "Strongly acidic or alkaline substances, for example with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested owing to their probable corrosive properties." With both the IRLG and OECD procedures, substances shown to be severe irritants in dermal irritation tests may be assumed to be eye irritants and need not be tested in the eye. The scientific bases for these exceptions are reviewed in the following sections.

The increasing number of studies on mechanisms underlying foreign compound-induced ocular irritation may yield a molecular approach to the prescreening of test agents based on a relationship of the physical/chemical characteristics of these agents with their specific biochemical/biophysical effects.

3.1.1 Corneal Penetration

Characteristics such as molecular size, configuration, osmotic pressure, and lipoidal nature, as well as chemical properties (including the degree of ionization following *in vivo* application) influence the capacity of an irritant to penetrate the tissues of the cornea. Factors affecting the penetration of substances into the rabbit cornea were investigated by Swan and White (1942). Rabbit eyes were exposed to test solutions of dye intermediates for three minutes, then rinsed with normal saline solution. After excision of the corneas, the test substances were isolated and coupled with other dye intermediates to form azo dyes which were measured colorimetrically. The results indicated that 1) penetration is inversely related to the degree of polarity of the molecule and 2) penetration rate is not inversely related to molecular size.

Cogan et al. (1944) studied the permeability characteristics of the beef cornea *in vitro*. Transfer of substances through the cornea was found to be primarily dependent on lipid/water solubility. While lipid solubility was necessary for substances to penetrate the corneal epithelium, water solubility was a requirement for penetration of the corneal stroma. Substances which penetrated both the epithelial cell layer and the stroma possessed properties of both lipid and water solubility. An approximate correlation was found between molecular size and penetrability through the corneal stroma, although penetration through the epithelium-stroma combination could not be correlated with molecular size. It was also found that charge or absence of charge alone could not account for differences in permeability.

Similarly, Cogan and Hirsch (1942) found that the penetration capacities of weak electrolytes through excised beef cornea were dependent on the lipid/water solubility of the dissociated and undissociated molecular forms. They concluded that corneal penetration is best achieved when the dissociation constant is sufficiently low so that the substance is present in the undissociated state at the hydrogen ion concentration of the eye, and when the undissociated form of the molecule possesses lipophilic characteristics.

The *in vitro* permeabilities to four organic liquids were studied in human and animal corneas by Marzulli (1965). Similar rates of penetration in the human cornea were found for dimethyl sulfoxide and 70% isopropyl alcohol (mean values: 53.4 and 53.1 micrograms/square centimeter/minute, respectively). Acetic acid (10%) and diethyl cresyl phosphate penetrated the human cornea at a slower rate (mean values: 1.6 and 3.0 micrograms/square centimeter/minute, respectively). Corneal penetration rates in the monkey, rabbit, dog, and swine, compared to man were found to vary with the agent tested, precluding an interspecies rank of corneal permeability.

In summary, 1) substances which possess maximum lipid and water solubilities and are poorly ionized have the greatest capacity to penetrate the cornea, and 2) the relative rates of corneal permeability among different species are a function of the specific agent tested.

3.1.2 pH

McLaughlin (1946), in a report of 500 cases of chemical eye burns resulting from accidental exposure, found that the most serious injuries were caused by highly acidic or basic materials.

Friedenwald et al. (1944) investigated the acid/base effect of a buffer solution in the rabbit cornea. Rabbit eyes were exposed to a solution containing buffer (sodium citrate, potassium acid phosphate, and boric acid, each at 0.028 molar, and sodium chloride at 0.038 molar) and hydrochloric acid or sodium hydroxide with a final pH ranging from 1 to 13. Solutions were administered by allowing one drop to fall directly onto the cornea every 2 seconds for a 10-minute period. Ocular lesions were graded on a numerical scale of 0-40 and were reported as the percentage of a maximum reaction (100%). The results showed a sharp rise in the percent reaction when the pH of the solution was greater than 11.5. A 100% reaction was observed with a pH of approximately 13. At the acid extreme, the increase in percent reaction per decrease in pH was less pronounced. The reaction increased from approximately 3% to 33% as the pH of the solution decreased from 3 to 1.

The above results in corneas with intact epithelium were compared to the effects produced on the corneal stroma following intracorneal injection of the test solution or exposure of the cornea to the solution after mechanical removal of the epithelium. At the alkaline extreme, no differences were found between effects on the intact cornea irrigated with test solution and effects on the corneal stroma. This finding is in keeping with the observed effects of swelling and desquamation of the corneal epithelium following irrigation with solutions of sodium hydroxide. A marked protective influence of the epithelial cell layer, however, was evident in tests at low pH. The percent reaction in the corneal stroma increased from approximately 5 to 70 as the pH of the solution decreased from 5 to 1.

Carpenter and Smyth (1946) determined the pH of the surface and aqueous humor of rabbit eyes exposed to 1.0% sodium hydroxide or a 5.0% aqueous solution of ethylene diamine. The treated eyes were irrigated with physiological saline for 30 minutes prior to the measurement of pH. With sodium hydroxide, the pH measurement of the aqueous humor of the treated eye was 8.5 whereas a value of 8.2 was found for the normal eye. The surface pH determinations of the treated and normal eyes were 8.0 and 7.2, respectively. With the less highly ionized alkali, ethylene diamine, values were: surface pH, 8.1; pH of aqueous humor, 8.7. The increased alkalinity of the surface and aqueous humor of the treated eyes indicates that alkaline materials can bind to and penetrate the rabbit cornea. Damage to internal ocular structures may be incurred where the buffering capacity of the aqueous humor is exceeded by the alkalinity of materials contacting the eye.

In contrast to the above determination of pH 8.2 for aqueous humor of the rabbit eye, Best and Taylor (1943) report a value of 7.1 to 7.3 for the corresponding human tissue. If reliable, these data present a 10-fold decrease in hydroxyl ion concentration which may have an important bearing on the respective responses of the human and rabbit eye to chemicals with pH-dependent ionization constants and, therefore, on the interpretability of rabbit eye data when these agents are tested.

Carpenter and Smyth (1946) suggested that alkalies are initially adsorbed to the cornea and subsequently penetrate into previously unaffected tissue resulting in a progressive burn. Severe lesions frequently develop as late complications although the injury may initially appear mild (Hughes, 1946). The degree of severity depends more on the pH of the solution and the duration of exposure than on the nature of the cation. In contrast to the effects of exposure to alkalies, no evidence for a similar progressive action of acid has been observed (McLaughlin, 1946). The early assessment of an acid burn is a measure of the long-term damage to be expected (Potts and Gonasun, 1975). Friedenwald et al. (1946) showed that the severity of an acid burn is dependent on both the pH of the solution and the protein affinity and protein precipitating ability of the anion. The severity of reaction produced by several acids of varying pH adjusted with sodium hydroxide was studied in the rabbit cornea following intracorneal injection. Ocular lesions were expressed on a scale of 0 to 100. With trichloroacetate solutions, as the pH decreased from 3 to 2, the reaction grade increased from 0 to 40. With chlorine solutions, decreasing the pH from 3 to 1 produced increases in the severity of reaction from grade 0 to 86. Decreases in pH from 5 to 1 with a buffer solution (citrate-phosphate-borate) produced increases in reaction from grade 0 to 74. With solutions of metaphosphate and sulfosalicylate, the reaction grade increased from 0 to 48 as the pH decreased from 7 to 1. Picrate, tungstate, and tannate solutions each produced somewhat uniform reactions from pH 9 to 1. Irrigation of the intact corneal epithelium with the same solutions produced reactions of much less severity.

Similar to the findings of Friedenwald et al. (1944, 1946), an investigation by Krueger (1959) showed that the corneal epithelium was a principal barrier to the penetration of acids. The penetrabilities of acids and alkalies were determined by measuring the pH of the anterior chamber by an implanted electrode following topical application to the cornea in isolated pig eyes. In eyes with intact epithelium, alkalies penetrated to the anterior chamber at a much faster rate than did acids. When the epithelium was removed, the penetration rates of acids and alkalies were approximately the same.

According to Grant (1974), splashes of concentrated strong acids, such as sulfuric, hydrochloric, nitric, phosphoric, and chromic acids, and liquid sulfur dioxide, can be as injurious to the eye as splashes of strong alkalies. He further states, however, "In tests on normal rabbit eyes, acids are found significantly injurious only when the pH is as low as 2.5 or lower. Applied to human eyes, solutions from pH 7 down to pH 2 induce an increasingly strong stinging sensation, but on brief contact cause no damage."

Guillot et al. (1981) studied the ocular irritation produced by 56 test substances, representing various chemical classes, according to the methods published by the *Journal Officiel Francaise* and the OECD, and the method proposed by the Association Francaise de Normalisation (AFNOR). Specific tests were conducted to validate the provision (OECD, AFNOR) that excludes the testing of substances with pH 2 or less. Measurements of pH were performed on the test agents; the pH of solid materials was measured from a saturated solution of the pulverized substance in distilled water. Four highly acidic substances (one liquid and three solids) received the highest test rating: extremely irritating. These were dimethylsulfate, pH 1.0; copper nitrate, pH 1.2; aluminum nitrate, pH 0.8; and oxalic acid, pH 1.0. One solid product with a pH of 2.1 (2,4-dichlorochlorosulfonyl-5-benzoic acid) was also extremely irritating while three solids with pH values ranging from 2.5 to 2.7 varied in irritancy. The OECD and AFNOR methods also specify that no test is required for substances with pH 11.5 or greater. Although no substances at this alkaline extreme were included in the study, Guillot et al. reported that two substances with a pH of 10.7 and one with a pH of 10.8 were very irritating, and one substance with a pH of 10.5 was extremely irritating.

The effect of pH on sensory irritation (stinging) has also been studied. Laden (1973) attempted to relate the stinging potential of acidic materials on human and animal skin to specific properties of the test agents. Stinging potential in the rat was measured as the length of time the abraded tail of the animal could be maintained in a test formulation before being flicked out. Effects in humans were evaluated by applying a small quantity of the test formulation to forearm skin which had been abraded by repeated stripping with tape. No general correlations were found between the stinging potential of solutions of acidic materials and the hydrogen ion concentration, the tonicity, or the nature of the anion. The sensory irritation of the eye to a specific solution containing primary, secondary, and tertiary sodium phosphates (iso-osmotic with the lacrimal fluid) at varying pH was studied in humans by Trolle-Lassen (1958). Statistical analysis of the results showed no irritation effects from the solution at pH values ranging from 7.4 to 9.6. The incidence of irritation increased from 1.0% to 99% as the pH decreased from 7.3 to 5.9 and as the pH increased from 9.7 to 11.4.

The above studies with alkali support the provision in the OECD guidelines that precludes the need to test substances of pH 11.5 or greater. Similar support is not unequivocally apparent for the OECD proposed cut-off of pH 2 or less, although the evidence suggests that this is at least in the right range.

3.1.3 Results of Skin versus Eye Irritation

Comparison of skin and eye irritation data indicates that substances which produce irritant effects on skin generally are also found to be irritating to the eye. In most cases the severity of irritation produced in the eye is similar to or greater than that observed in skin. For example, of 195 chemicals given relative hazard ratings for both ocular and dermal (no patch) irritation in rabbits, 45% were rated as more hazardous to the eye than to the skin, 44% were given the same numerical rating for the severity of both types of irritation, and 11% were rated as more hazardous to the skin than to the eye (Sunshine, 1969). Three of the 22 compounds comprising the latter group (bromomethane and two mixtures of chlorinated biphenyls) showed the greatest deviation from the normal pattern in that the relative hazard grades indicated markedly greater irritation to the skin than to the eye under the conditions of the tests. In every case, however, a compound found to be irritating to the skin was also observed to produce a positive response in eye tests.

Similarly, in tests of 6 shampoos, Jacobi and Ortmann (1971) found results of irritancy in the rabbit eye to parallel results for the closed patch test in rabbit skin.

Included in a table of range finding data for over 300 compounds are the scores for both primary skin irritation on the uncovered rabbit belly and rabbit eye injury (Smyth et al., 1962). While several compounds were found to be irritating to the eye but not the skin, the majority produced both types of irritation. A small percentage of compounds (ca. 4%), however, were found to be minimally irritating to the eye but were severely irritating to the skin.

Results of a toxicity survey of 145 detergents showed that all were eye irritants, whereas 47% also were either primary skin irritants or corrosives. (Seabaugh et al., 1977).

The above findings, as well as the generally recognized relationship between the effects of chemicals on skin and mucous membranes, support the IRLG and OECD guidelines provision that substances found to be severely irritating in dermal irritation tests are assumed to be ocular irritants and, therefore, need not be tested in the eye.

3.2 TEST AGENT VOLUME

The original Draize test specifies the instillation of 0.1 milliliter of the test agent into the conjunctival sac of the rabbit eye. The procedures of the FHSA, IRLG, and OECD stipulate a volume of 0.1 milliliter for liquids and a volume equivalent to 0.1 milliliter or a weight of not more than 100 milligrams in the testing of solids. According to the test protocol recommended by the NAS, two or more different doses of the test material which are within the range of probable human exposure should be used. Suggested doses are 0.1 and 0.05 milliliters.

Griffith et al. (1980) studied the irritancy of several substances in the albino rabbit eye with the objective of producing a degree and persistence of irritation approximating that commonly found in human experience. Twelve test materials were each administered in volumes of 0.01, 0.03, and 0.1 milliliters of liquid or dry weight equivalent. Only hexane and 0.1% benzalkonium chloride produced no observable irritation at all tested volumes. The average maximum scores for the irritating materials were found to be lower for the smaller volumes of material, and eyes treated with the smaller volumes tended to clear completely in less time. Isopropyl alcohol, however, displayed a flatter dose/response pattern. The 0.01 milliliter dose was the most sensitive in separating the irritability indices of the materials for all observation periods except day 21, in which case 0.03 milliliter resulted in better separation. The test materials were classified as innocuous or slightly irritating to severely irritating or corrosive to the human eye, based on descriptions in the literature, occupational incidents, and reports of consumer exposures. When the evaluations for humans were compared with the experimental results for each test volume, the 0.01 milliliter dose data best matched human experience. While recognizing the importance of attempting to achieve greater precision and sensitivity in the assessment of irritation, the authors view accuracy in prediction of human responses as an even more important goal.

Bell et al. (1979) studied the effect of varying the percent activity of test materials rather than the volume instilled in the eye. Shampoos with approximately 15% active chemical (undiluted) usually produced moderate tissue damage when diluted to 2.5% to 3.0%, and this dilution more readily distinguished between preparations in terms of corneal and iridial damage than did no dilution or lesser dilutions. Concentrations of at least 7% active matter accentuated changes and masked differences in irritant potential which had been detected at lower concentrations.

Use of the standard volume (0.1 milliliter) has historically provided reliable detection of the adverse effects produced by test agents. Recent evidence suggests, however, that the use of smaller volumes (e.g., 0.01 milliliter) or appropriate dilutions of test agents can increase the ability of the test to differentiate between the irritancy potential of different substances. For substances which require extensive characterization, testing with additional animals to assess the effects of smaller volumes or dilutions may thus provide useful supplementary information.

3.3 METHOD OF EXPOSURE

3.3.1 Liquids and Solids

The procedures specified by Draize, the FHSA, IRLG, and OECD call for the direct instillation of the test agent into the lower conjunctival sac of the rabbit eye. In a survey of individuals involved in testing products for potential eye irritation, Beckley (1965b) reported that the sprinkling of a powder or granular substance on the surface of the cornea rather than directly introducing it into the lower conjunctival sac was recommended as more closely simulating accidental entry into the human eye. According to the NAS, application to the eye should be conducted in a manner that reflects the probable mode of accidental exposure. When direct application is called for, the material should be dropped or sprinkled directly onto the cornea rather than being instilled into the lower conjunctival sac. The lids should be held open momentarily to ensure contact of the substance with the cornea, then gently released. While direct corneal application may be justified at times in the assessment of specific hazards, the overall effectiveness of conjunctival sac instillation supports its continued use as the standard procedure.

3.3.2 Aerosols

The test guidelines of the IRLG and OECD include a specific provision for the testing of aerosols. Aerosol products are administered in a single, short burst for about one second at a distance of 10 centimeters (OECD) or about four inches (IRLG) directly in front of the eye. This provision responds to a criticism reported by Beckley (1965b) concerning the testing of aerosols in which 0.1 milliliter of the collected aerosol liquid is directly instilled into the conjunctival sac.

Spray exposure to aerosols may cause eye damage not only due to the active ingredients, but also to the physical impingement of the particles as well as the cooling produced by the propellant (Idson, 1968). MacLean (1967) reported cases in which accidental exposure to household sprays resulted in an "aerosol keratitis" (corneal reaction to the impregnation of the epithelium with small particles by the force of a pressurized chemical spray). This keratitis is generally delayed in onset, mild, and transient; although recovery from the more severe forms may take several months.

Giovacchini (1972) has reported using three different treatment groups of rabbits in testing aerosols. Two groups received the liquid concentrate. The eyes of one of these groups were subsequently washed. In the third group, the material was sprayed into the open eye for one second at a distance of six inches. Others have compared the results of spray exposure to aerosols with the results of direct instillation. Exposure of the rabbit cornea to several brands of hair sprays showed that all of the products were eye irritants when dispersed as a spray (details of exposure not given), while only some products were eye irritants following direct instillation of the collected hair spray liquid (Marzulli and Simon, 1971). Kay and Calandra (1962) have also examined the influence of exposure methods on results using aerosol hair sprays. Draize instillation of 22 products resulted in a mean score of 4.5, whereas spray instillation of 13 products at an unspecified distance from the eye produced a mean score of 3.5. The former value places the products in the classification of moderately to severely irritating, while the latter classifies them as mildly to moderately irritating. Since unequal numbers of unidentified products were tested by the two methods, it cannot be concluded that the differences in the mean scores and resulting ratings were due only to the method of exposure. MacLeod (1969) reported that spray exposure to tear gas in monkeys (into the open eye for two seconds at a distance of four centimeters or directly in front of the face for four seconds at a distance of six feet) produced effects of less severity and persistence than direct instillation. Further knowledge of the manner in which humans are exposed to aerosol sprays may be of help in selecting the appropriate procedures for the testing of these products.

3.3.3 Corneal Applicators

Devices have been used to direct the administration of test material to the cornea and exclude it from contact with the conjunctiva. In a comparative evaluation of eye irritation in monkeys and rabbits, Buehler and Newmann (1964) found that the use of a cup aspirator in rabbits produced lower scores in tests with surfactant formulations than those that were obtained by instillation into the conjunctival sac. These scores more closely approximated the response seen in monkeys treated by direct instillation onto the cornea. On the other hand, the application of 1% sodium hydroxide with a cup aspirator in rabbits produced a more severe corneal opacity (with permanent impairment of vision) compared with the effect produced by instillation into the conjunctival sac. Battista and McSweeney (1965) demonstrated that the use of a corneal applicator in rabbits produced a greater and more uniform corneal opacity and less variability between tests when compared with instillation of the test agent into the conjunctival sac. A viscous solution of strong alkali was used to determine the effective time required to produce an opacity score of 2 after seven days. The result following conjunctival sac instillation was 7.75±2.5 (mean ± S.D.) seconds compared with 3.9 ±0.87 seconds for applicator instillation. Although the detection of irritants requires the evaluation of damage to the whole eye and not to the cornea alone, the carefully controlled exposure provided by application devices may be useful at times for characterizing specific corneal effects.

3.4 DURATION OF EXPOSURE

The role of the length of exposure and the efficacy of flushing the eyes with water following treatment in modifying and ameliorating the severity of the irritation reaction are determined when the original Draize test methods are used. Floyd and Stokinger (1958) found that the severe irritation produced by several organic peroxides (methyl ethyl ketone peroxide, cumene hydroperoxide, and t-butyl hydroperoxide) could be prevented in all cases by washing the eyes of the rabbits with water four seconds after instillation of the test solutions. Olson et al. (1962) demonstrated that washing within 30 seconds after application reduced the severity of reaction to surfactants in the rabbit eye.

Gaunt and Harper (1964), using the Draize methodology, studied the potential irritancy of 10 commercially available shampoos. Treatment with each sample was as follows: sample instilled, no further treatment; 10% dilution of sample instilled, no further treatment; sample instilled, eye irrigated with 20 milliliters water at two seconds; and sample instilled, eye irrigated with 20 milliliters water at four seconds. Dilution of the sample with water prior to its instillation into the eye resulted in marked decreases in the scores. While irrigation provided an ameliorative effect in most cases, in one case a more severe effect was produced; one shampoo induced severe corneal opacity in two out of six rabbits when the eyes were irrigated with water. Little difference in benefit was observed between the two and four second washing procedures. These results show that a test protocol using one group of animals which receive the undiluted test agent with no further treatment and another group of animals which have their eyes washed at a fixed interval following exposure to the test material may provide valuable information on the eye irritation capacity of test materials.

Guillot et al. (1981), in the study mentioned earlier, performed tests with irrigation according to the OECD protocol, whereby substances found to be irritating may subsequently be tested in separate groups receiving irrigation at 4 and 30 seconds after instillation. Twelve of 23 substances rated irritating to very irritating received lower classifications of irritancy with rinsing at 30 seconds. For eight of the remaining 11 substances for which rinsing at 30 seconds produced no classification change, a lower classification of irritancy resulted when rinsing was initiated at four seconds. For 7 of 15 severely to extremely irritating substances, rinsing at 30 seconds resulted in a lowering of classification. With four of these seven substances, rinsing at four seconds produced a further lowering of classification.

A modification of the Draize test procedures was proposed by McDonald and Shadduck (1977) for evaluation of "dermatologic type products." Both eyes of albino rabbits are used, three rabbits comprising each test group. Irrigation with 200 milliliters of tap water for one minute follows treatment at 20 seconds, 5 minutes, and 24 hours using separate test groups. Six sham control eyes each are washed at time 0 and at 24 hours. An additional six eyes serve as untreated controls and a commercial product control is included among the test materials. Eyes are examined and graded by a slit-lamp scoring system at 1, 24, 48, and 72 hours and on days 7 and 14; the test may be terminated at any point if all treated eyes have returned to normal. The wash times were selected because ocular changes for a variety of products have been found to differ depending on the length of exposure prior to washing. Of 75 shampoos tested, 80% produced ocular reactions of greater severity, higher incidence, and longer duration in animals undergoing irrigation at 5 minutes following instillation compared with those undergoing irrigation at 20 seconds or 24 hours. In experiments with a skin cleanser, washing at 20 seconds following exposure entirely prevented the irritation observed at wash times of 5 minutes and 24 hours following exposure. The early washing, however, resulted in more severe conjunctival congestion with an acne scrub product.

Bayard and Hehir (1976) investigated different durations of exposure to test agents in the rabbit eye irritation test. Twelve rabbits per treatment group were exposed to an acid, base, or alcohol for five minutes. An additional 12 rabbits were exposed for 24 hours. Results showed that the effects of a 5-minute exposure were not significantly different from those for a 24-hour exposure at the 24-hour examination, but were significantly less severe for opacity, iritis, chemosis, redness, and overall scoring when examined at 48 and 72 hours.

Davies et al. (1976) investigated the maximum delay time for remedial irrigation in the rabbit eye following instillation of a 10% solution of sodium lauryl sulfate. Corneal opacity or a dulling of the normal corneal luster was produced in every rabbit when the delay between instillation and washing was 20 seconds or longer. Eyes exposed to the test agent for 20 or 30 seconds displayed reactions that were similar to those in eyes exposed for up to two minutes and only slightly better than the reactions observed following no irrigation. Reactions were fewer and less severe with exposure times under 20 seconds. No corneal opacity was produced after four seconds exposure although a dulling of the corneal luster was observed in four animals. The range of critical exposure before corneal damage is produced in the rabbit eye after instillation of a 10% solution of sodium lauryl sulfate was, therefore, concluded to be 4-10 seconds. Irrigation with 100 milliliters of water produced no further reduction in the irritant response observed with a 20 milliliter wash volume.

Using sodium hydroxide, acetic acid, and hydrochloric acid, Battista and McSweeney (1965) investigated ocular irritation reactions as a function of exposure time in rabbits. Appropriate rinsing schedules and a corneal applicator were used, varying exposure conditions quantitatively between no-effect and maximum-effect levels. From the resulting time response curves, the relative irritancy of the different samples could be compared by estimating the exposure time required to produce equivalent lesions. The following concentrations and approximate exposure times before rinsing produced an estimated opacity score of grade one at the seven-day observation point: sodium hydroxide: 1.0% at 26 seconds; acetic acid: 21% at 2 seconds, 10.5% at 28 seconds, and 5.25% at 65 seconds; and hydrochloric acid: 3.7% at 9 seconds and 1.9% at 70 seconds. With this method, interpretation and comparison of data are reduced to a single time measurement for achieving whatever level of response is chosen. In addition, an estimate of the maximum exposure time that will be tolerated by the eye for a given test material can be obtained.

Seabaugh et al. (1976) compared 32 chemicals for effects of length of exposure with a two-minute irrigation period. Irritancy changes, recorded during a seven-day observation period, were compared for exposure times ranging from 30 seconds to 24 hours. For 30-second versus 24-hour exposures, decreases in irritancy were observed with 20% of the chemicals, increases with 5%, and no change with 75%. In comparisons of 2-minute and 24-hour exposures, 15% decreased, 10% increased, and 75% showed no change. For 5-minute compared with 24-hour exposures, 13% decreased, 10% increased, and 77% did not change. For six of the chemicals tested, both a 2-minute and a 15-minute irrigation was evaluated. In general, there were no apparent benefits from washing rabbits eyes for either 2 or 15 minutes at any of the shorter chemical exposure times as compared with the 24-hour exposure. According to the authors, these data indicated that irrigation of rabbit eyes does not contribute to the outcome of eye irritancy tests in most cases when compared with the official FHSA procedure.

Gupta and Schiavo (1976) have reported a rapid testing procedure in rabbits which differentiates low levels of ocular irritation by multiple exposures to test agents. Two drops of a test solution are instilled into the lower conjunctival sac of either eye hourly, nine times a day for four consecutive days, according to a balanced incomplete block design. Two different solutions are tested in each animal, using one eye for each material. With this method, 10 rabbits were used to test four ophthalmic formulations of benzalkonium chloride in different concentrations. Normal saline was included as a control. Each of the 10 possible combinations of the five formulations occurred once. Eyes were examined before treatment and before the first instillation on each day of treatment. Ocular reactions were graded by the FHSA method of assessment with the addition of conjunctival discharge scores. Gross observations and a statistical analysis of the data revealed that the formulation containing the highest concentration of benzalkonium chloride caused the most ocular irritation. Previous testing of this solution in rabbits using standard laboratory procedures and the Draize method of grading ocular irritation failed to detect the reactions. Thus, the repeated instillation of ophthalmic formulations permitted an evaluation of irritancy with particular relevance to human exposure conditions.

The FHSA, IRLG, and OECD methods modified the original Draize procedure by changing post-treatment washing from a requirement to an optional wash after the 24-hour observation period. The OECD protocol further states that additional tests employing irrigation may be indicated for certain substances found to be irritating. In these cases, the eyes of six rabbits are washed for five minutes using a volume and velocity of flow which will not cause injury. Three of the rabbits receive irrigation at four seconds after instillation; the other three rabbits at 30 seconds. According to the NAS procedures, irrigation is similarly not required for determining the inherent irritancy of a substance.

3.5 SELECTION OF SPECIES

The use of the rabbit in eye irritation testing has been recently criticized by animal welfare groups. The rationale for and problems with this species selection as compared with other alternatives are reviewed in the following sections.

3.5.1 Use and Characteristics of Different Species

The albino rabbit is the test animal specified in all eye irritation testing guidelines and regulations for a number of reasons. The animal itself is readily available, docile, easily handled, relatively inexpensive, and easy to maintain. The rabbit eye is large and the corneal surface and bulbar conjunctival areas are both large and easily observed. The iris is unpigmented, allowing ready observation of the iridial vessels (McDonald and Shadduck, 1977). There is also a wealth of comparative irritancy data that has been generated using rabbit eyes. The large conjunctival sac offers a convenient "pocket" for instillation of a measured volume of test material. On the other hand, limited comparative data from controlled exposures of humans and rabbits generally show that responses of the rabbit eye are much more severe and long lasting. There are also several examples of cases in which rabbit tests have failed to alert investigators to effects that appeared later in humans. Rieger and Battista (1964) described cases in which the Draize test erroneously classified a widely distributed shampoo as irritating and failed to predict the inherent danger in the use of a neutralizer or to select the milder (by human assessment) of two relatively mild products.

The rabbit eye possesses several anatomical and physiological differences from the human eye which may contribute to differences in response. The cornea is thinner, Bowman's membrane, if it exists at all, is essentially unrecognizable, there is a well developed nictitating membrane, the fur surrounding the eye and eye lids is thick, the blink reflex is not well developed, pH of aqueous humors are different (rabbit: pH 8.2, human: pH 7.1-7.3), the rabbit eyelids are looser, and the tearing mechanism is less effective (NAS, 1977; McDonald and Shadduck, 1977). In addition, despite the general marked improvement in standardization of laboratory animals, there are still significant variations among laboratory rabbits. Weltman et al. (1965) have commented that age, strain, and sex differences may be important in ocular irritation tests, although no specific data were presented.

In an effort to improve the reliability of tests in predicting human eye irritation, other species have been examined including the guinea pig, rat, mouse, hamster, chicken, dog, cat, and monkey. With the possible exception of the monkey, none of these species has been studied thoroughly enough yet to substitute for the rabbit as the standard test species.

Among non-human primates, the rhesus monkey has been used most frequently. Rhesus monkeys, however, are expensive, difficult to obtain, and are even less uniform than rabbits. Handling and restraint can be difficult and their pigmented irises complicate the observation of some irritation responses.

The corneal thickness of different animal species varies considerably (Table 3). These differences in thickness are important, since the capacity of an irritant to penetrate the cornea is inversely related to total corneal thickness (Marzulli and Simon, 1971). Corneal penetration rates of various substances *in vitro* have shown interspecies variations (Marzulli, 1965). Kuhlman (1959) has shown species variation in the enzyme concentrations of rabbit, cat, and rat corneal epithelium.

Table 3. Corneal Thickness of Several Species of Animals

Species	Thickness (Millimeters)	Reference
Cat	0.62	Marzulli and Simon (1971)
Dog	0.55	Marzulli and Simon (1971)
Rhesus Monkey	0.52	Marzulli and Simon (1971)
Rabbit	0.37	Marzulli and Simon (1971)
Mouse	0.10	Davson (1962)
Human	0.51	Maurice and Giardini (1951)

Recognition of the need for additional or alternate animal models in eye irritation testing is illustrated by the criteria which have been suggested for determining the significance of ocular irritation in the assessment of dermatologic products (Aronson, 1975). The author states that an irritation reaction classified as significant (any ocular irritation reaction which leads to or can potentially lead to permanent or functional change) requires evidence of irritation in both humans and an animal species. When human data are not available, presumptive evidence may be demonstrated in two different animal species. Initial testing should be performed in the albino rabbit and, when necessary, analogous studies should be conducted, preferably in a primate. Similarly, the NAS (1977) recommends the use of the monkey as a second test species when confirmatory data are required.

While the rabbit eye is characterized by certain differences in anatomy and physiology in comparison with the human eye, it also possesses functional attributes which provide a reliable system for assessing the irritant properties of chemicals. The generally greater responsiveness of the rabbit eye increases the possibility of detecting compounds which are eye irritants and provides for a conservative risk assessment. Continuing research is needed, however, on alternate species and test methods for better characterizing substances which pose special hazards to man.

3.5.2 Comparison of Monkey and Rabbit Responses

Similarities in the anatomy and physiology of the monkey and human eye suggest that the monkey might predict more accurately than the rabbit model the potential of substances to produce eye irritation in humans. Reactions to eye irritants have, therefore, been compared in the monkey and rabbit (and in some cases, in humans). The eye irritation responses of the rhesus monkey and the albino rabbit were compared in a comprehensive study by Buehler and Newmann (1964). Four materials were tested by direct instillation into the conjunctival sac of the rabbit eye and onto the cornea of the monkey eye. Test substances were the cationic surfactant benzalkonium chloride, a liquid anionic surfactant formulation (37% coconut alkyl-sulfate, 12% coconut ethanolamide, and 18% ethanol), a nonionic/cationic surfactant mixture (20% nonylphenoxypolyethyleneoxy ethanol and 2% lauryl isoquinolinium bromide), and sodium hydroxide. The rabbit eye was also tested by application of these same materials with a cup aspirator to expose the corneal surface without subjecting the bulbar and palpebral conjunctiva to a corresponding insult. Resulting corneal effects in the monkey after direct instillation were restricted to superficial changes such as physiologic edema and slight surface alterations, whereas instillation into the rabbit conjunctival sac produced a hyper-response characterized by varying degrees of opacity, pannus formation, and tissue buildup. With the exception of sodium hydroxide, the response of the rabbit eye more closely approximated the response of the monkey eye when the cup aspirator was used. Differences in species response were attributed to anatomical/physiological differences and to the effect of conjunctival irritation on corneal response.

A preliminary study comparing ocular responses in the rhesus monkey with those in albino rabbits and humans indicated that the rhesus monkey more nearly reflected human responses than did the rabbit (Beckley et al., 1969). Instillation of a 5% soap solution in rabbit eyes showed almost no corneal epithelial effect. The same material caused corneal epithelial damage in both monkeys and humans. While a detergent composition produced irritation in all three species, the rabbit response was greater than the human or monkey response.

Benke et al. (1977) observed that two surfactant formulations produced severe irritation when tested undiluted in rabbit eyes. Moderate irritation was produced when 10% dilutions were used. In monkey eyes, however, the formulations were found to be considerably less irritating even when undiluted and unrinsed.

A comparative study of responses of the rabbit and monkey eye was reported by Green et al. (1978). A number of substances were investigated in tests with and without irrigation. Reactions were graded throughout a 21-day observation period by external observation in addition to the use of a slit lamp scoring system. Responses to each substance were classified based on the most severe response that was seen in any one of a group of animals receiving the same treatment. Histological observation and photographic documentation of the ocular lesions were also performed. The rabbit eye was found to be more sensitive to injury than the monkey eye for most of the chemicals studied, the principal exception being 5% sulfuric acid. The difference in scores between the two species was greatest with 1% sodium hydroxide in which case the rabbit response was much greater than that of the monkey. The scores for the two species also followed different patterns with time. With most substances the monkey eye showed maximum response at one hour whereas the maximum response of the rabbit eye was observed at 24 hours. The difference in scores between the two species depended somewhat on the method of grading. Analysis of variance showed the general increase in the rabbit eye response to be greater compared with the monkey eye when scores were obtained using the slit lamp as opposed to external observation. No pattern was seen in the comparative responses of the two species to irrigation (at two minutes following instillation of the test agent), though rinsing generally produced lower scores. According to the numerical scores, rabbit eyes treated with 1% sodium hydroxide benefitted markedly from rinsing but rinsing was less beneficial to monkey eyes treated with the same agent. Classification of responses indicated that, in fact, irrigation produced a marked increase in the response of the monkey eye to a 5% solution of sulfuric acid. In the rabbit eye, however, irrigation lowered the classification of the response to the same agent.

Table 4 presents the comparative response of the rabbit and monkey eye to test agents. For most test substances, the degree of irritation produced in the rabbit eye is greater than that produced in the monkey eye. A comparison of animal and human responses is presented in Table 7. In general, the decreased response of the monkey eye compared with the rabbit eye more closely approximates the response found in humans. Testing in the monkey, therefore, may be valuable for predicting the degree of irritation produced by substances contacting the human eye. Use of the rabbit is advantageous for maximizing the probability of detecting substances that may be eye irritants for man.

3.5.3 Other Species

Except for studies of monkeys, apparently little effort has been made to establish and validate the use of species other than the rabbit in eye irritation testing. According to Grant (1974), guinea pigs and rats are less satisfactory than rabbits, though no details are given.

Sanderson (1959) reported using the guinea pig to assess ocular irritation as part of a screening process to evaluate a number of organic solvents and emulsifying agents for possible use as injection solvents in toxicity testing. A 10 microliter drop of the compound was placed on the corneal surface of one eye with the untreated eye serving as a control. No details were provided regarding time course of observation or whether any specific scoring system was used. The toxicity of glycerol formal was reported in detail. Administration of this agent to the eye of the guinea pig caused only slight temporary irritation without pupillary constriction or obscuration.

Leuenberger (1973) used the rat to study the severity of lesions and ultrastructural changes in corneal epithelium following topical application of various anesthetics. All of the drugs produced similar effects. Since others have observed non-uniformity in the effects produced by these agents in different test systems, the rat model apparently possesses limited discriminatory power.

The rat model was studied for sensitivity to three comercial products established as irritants by the Draize rabbit eye test (W. Troy, personal communication, 1981). In the rat eye all three products were found to be non-irritating. Mel'nikova and Rodionov (1979) found that the rabbit eye was more sensitive than the eye of the rat or guinea pig to the irritancy produced by the antibiotic, grisein. Rat eyes treated with 20% to 30% solutions of the antibiotic returned to normal at seven days,

Table 4. Comparison of Response of the Rabbit and Monkey Eye to Irritants

Test Agent	More Sensitive Species	Reference
Surfactant formulations	Rabbit *(a)*	Buehler and Newmann (1964)
1% Sodium hydroxide	Rabbit	Buehler and Newmann (1964)
Cytarabine hydrochloride	Similar *(b)*	Elliot and Schut (1965)
Liquid detergent	Rabbit	Beckley (1965a)
Various materials	Rabbit	Carter and Griffith (1965)
5% Soap solution	Monkey	Beckley et al. (1969)
Detergent	Rabbit	Beckley et al. (1969)
Chloroacetophenone	Rabbit *(c)*	MacLeod (1969)
Iodine solution	Rabbit	Hood et al. (1971)
NS *(d)*	Rabbit	Giovacchini (1972)
Surfactant formulations	Rabbit	Benke et al. (1977)
Commercial shampoos, cationic detergents	Rabbit	Gershbein and McDonald (1977)
Variety of substances	Rabbit	Green et al. (1978)
5% Sulfuric acid *(e)*	Monkey	Green et al. (1978)

(a) Response of the rabbit to test agent instilled with a corneal applicator more closely approximated the monkey response.

(b) Peak effects were observed in rabbits at 7 days and in monkeys at 8-12 days.

(c) In some cases, the two species showed similar effects.

(d) Not specified.

(e) Eyes of both species were irrigated.

but reactions in rabbit eyes persisted for three weeks following instillation of a 10% solution. A 30% solution in guinea pig eyes brought about a progressive clouding of the cornea and areas of corneal opacity. Because the main objective of the study was not the comparison of ocular responses in different species, these comparisons were not fully reported.

An evaluation of the corneal irritancy of four commercial shampoos and two cationic detergents in various animal species was conducted by Gershbein and McDonald (1977). The following order of decreasing corneal sensitivity to the shampoos was inferred from the findings: rabbit, hamster, mouse, rat, guinea pig, dog, cat, rhesus monkey, and chicken. The mouse and hamster corneas appeared to be more sensitive to the two detergents than the rabbit, the sensitivity of the guinea pig and rat corneas was lower, and the remaining species appeared less sensitive although the results were based on only a small number of eyes. The corneal lesions of the albino rabbit in the above tests were generally more persistent and cleared with greater difficulty compared with those of the other larger species. The rabbit also displayed the highest sensitivity to conjunctival damage by the various test agents among the larger species examined. The authors suggest that current eye data from the various species may not be strictly relevant to man, and they recommend caution in extrapolating rabbit-eye findings to human experience.

Results of eye irritation tests with a liquid detergent in the rabbit, dog, monkey, and man were reported by Beckley (1965a). Corneal reaction was found to be slightly greater in the dog than in the rabbit, with the monkey cornea showing the least sensitivity. Iridial and conjunctival sensitivity was greatest in the rabbit, and least in the monkey. Results of three separate studies in humans with the same test agent showed that the irritation response was characterized by varying degrees of conjunctivitis. No effects were found in human iris or cornea.

Giovacchini (1972) observed that proposed products which were irritating in the standard rabbit eye test were less irritating when tested in monkeys and dogs. Studies in humans showed that the irritation responses more closely resembled those of the monkey and dog rather than the rabbit.

The sensitivity of various species to eye irritants is summarized in Table 5. In view of the generally greater response to ocular irritants in the rabbit compared with other species, including man, it would seem reasonable to further explore the predictive value, discriminatory power, and practicality of other animal test species.

Table 5. Comparison of Response of Several Species to Eye Irritants

Test Agent	Decreasing Order of Sensitivity of Species Tested	Reference
Liquid detergents	Rabbit; Dog *(a)*; Monkey	Beckley (1965a)
NS *(b)*	Rabbit; Dog, Monkey	Carter and Griffith (1965)
NS	Rabbit; Dog, Monkey	Giovacchini (1972)
Commercial shampoos	Rabbit; Hamster; Mouse; Rat; Guinea Pig; Dog; Cat; Monkey; Chicken	Gershbein and McDonald (1977)
Cationic detergents	Mouse, Hamster; Rabbit; Guinea Pig, Rat; Dog, Cat, Monkey, Chicken	Gershbein and McDonald (1977)
Grisein	Rabbit; Guinea Pig, Rat	Mel'nikova and Rodionov (1979)

(a) Corneal effects were greatest in the dog.

(b) Not specified

3.6 TEST GROUP SIZE

The choice of test group size involves balancing economic considerations and practicality with the benefits of using large test populations for increased accuracy and reliability of test results. Weltman et al. (1965) demonstrated that considerable differences may occur between irritation scores of different sized groups. As the population decreased from 24 to 4 animals, the Draize scores showed a greater degree of variability and spread. The authors suggest that increasing the test group size in the standard Draize procedure would help avoid chance classification errors when small populations are employed.

Results of the study by Bayard and Hehir (1976), in which 12 rabbits comprised each treatment group, indicated that a reduction in the number of animals would decrease the ability of a test to differentiate degrees of irritation since a large variability between individual rabbits was noted. Heywood and James (1978), however, point out that while reducing the number of animals would decrease the ability of the test to differentiate degrees of irritation, increasing the group sizes would not increase precision in scoring the ocular reaction. Nonetheless, accuracy in ranking the irritancy of test substances evidently could be improved by using larger numbers of animals, particularly for borderline responses.

Guillot et al. (1981) compared the mean ocular irritation ratings in two groups of three rabbits each with those obtained using six rabbits. Classification differences due to test group size resulted for 25 of 56 substances tested. In only two cases, however, was a test substance classified as non-irritating based on results in one group of three rabbits while testing with three additional rabbits and with six rabbits resulted in a rating of "slightly irritating." Thus the data showed that the use of three animals in a preliminary test was adequate in differentiating a positive from a negative response for roughly 96% of a wide variety of substances. The importance of test group sizes in evaluating ocular irritancy, therefore, appears to have been adequately addressed in the methods currently required for regulatory purposes as well as those which have been recommended.

The FHSA method reduced the Draize requirement from nine to six animals per group. According to the IRLG and OECD, initial testing should be performed on at least three animals, but additional animals may be required to clarify equivocal responses. The NAS procedures recommend that a minimum of four animals be used per dose level, unless unequivocal evidence of severe irritation or corrosivity can be obtained using a smaller test group.

3.7 USE OF ANESTHETICS

The purpose of using anesthetics during eye irritation testing is to reduce unnecessary pain and stress to the experimental animal without altering the test results. While historically ocular anesthetics have not been used, investigations into the use of these drugs have begun in recent years in response to the increasing concern for the humane treatment of test animals. Although it does not specifically preclude their use, the FHSA testing procedure makes no provision for the use of anesthetics. Both the IRLG and OECD guidelines provide the option to use a local anesthetic prior to the instillation of the test substance.

Concern has been expressed that the use of anesthetics may interfere with the test results. A comment received in response to the publication of the IRLG guidelines suggests that the use of anesthetics could inhibit or reduce a normal blink reflex. This could cause corneal drying and result in irreversible damage from a test agent. According to Grant (1974), local anesthetics increase the permeability of the corneal epithelium to drugs and chemicals.

Four topical ocular anesthetics were studied in the rabbit eye by C. Hoheisel (personal communication, 1981). Effects of a number of irritants were compared in the presence and absence of ocular anesthesia induced by proparacaine hydrochloride, tetracaine hydrochloride, benoxinate, and lidocaine. Whereas 0.5% sodium hydroxide caused only conjunctival redness without iritis or opacity in control eyes, it produced corneal opacity in proparacaine-treated eyes. The use of proparacaine resulted in the reclassification of a 10% liquid dishwashing detergent solution from the nonirritant to irritant category under FHSA regulations. With other test agents, proparacaine did not affect the irritation scores sufficiently to require reclassification as eye irritants. Lidocaine did not sufficiently relieve pain and benoxinate interfered with test scores. When dosed in a single application, tetracaine did not adequately raise the pain threshold. When dosed in two applications 10-15 minutes apart, however, its pain relief properties improved and the test scores were not altered.

Butacaine sulfate was studied in rabbits by Ulsamer et al. (1977). Parameters measured following instillation of test agents to anesthetized and unanesthetized eyes included opacity, corneal water content, dry weight, and electrophoretic protein patterns. No significant differences in dry weight or in corneal electrophoretic protein patterns were noted with any irritants tested. While neither opacity scores nor moisture content produced a clear pattern of statistically significant differences for anesthetized versus unanesthetized corneas, the following effects were observed. With 10% acetic acid and 10% ammonia, the unanesthetized eyes had lower opacity scores. Following the administration of 5% acetic acid and 1% ammonia, the anesthetized cornea had a higher water content. Anesthesia produced a lower water content with 3% sodium hydroxide.

Using 10% sodium lauryl sulfate as the irritant and 0.5% proparacaine as the anesthetic in rabbits, Heywood (1977, as cited in Heywood and James 1978), showed that no statistically significant differences could be detected between the anesthetized and unanesthetized cornea. The intensity of the reaction apparently increased following anesthesia but there was no evidence of prolonged effect. In the monkey, anesthesia had a very marked effect on irritants in which all reactions were intensified and prolonged. The results indicated that use of a local anesthetic is feasible when carrying out eye irritation tests in rabbits, but is contraindicated in the monkey. In contrast to the findings of Heywood, Bell et al. (1979) have reported the tendency of anesthetic use to delay recovery from ocular damage in the rabbit, although the specific type of anesthetic used was not identified.

Studies of the effects of anesthetic agents alone on the eye may contribute toward identifying an anesthetic suitable for ocular irritation testing. Gundersen and Liebman (1944) reported the effects of a number of topical anesthetics on the regeneration of the guinea pig corneal epithelium. The anesthetics were repeatedly administered to the eyes following mechanical abrasion of the cornea. Of the anesthetics tested, 0.5% tetracaine hydrochloride and 1.0% phenacaine hydrochloride were the least toxic to the regenerating epithelium. The inhibitory effect of 0.5% tetracaine was almost entirely eliminated by increasing the tonicity of the solution in which it was administered. Tests with other anesthetics showed an increasing inhibitory effect in the following order: 1.0% butacaine sulfate, 4.0% larocaine hydrochloride, 4.0% cocaine hydrochloride, and 10% cocaine hydrochloride. The effects were also studied in cats and rabbits, but, according to the authors, the results obtained in these species were equivocal. It was concluded that the concentration and tonicity of anesthetic agents can exert a significant effect in the corneal wound healing process.

In a similar study, Bykov and Semenova (1972) showed that 2.0% lidocaine caused less delay in epithelial healing than 2.0% cocaine or 0.5% tetracaine in the rabbit cornea. Pfister and Burstein (1976) reported that no disruptive effects were observable by scanning electron microscopy following single-dose application of 0.5% proparacaine or 0.5% tetracaine to rabbit eyes. A preparation of 4.0% cocaine, however, produced significant plasma membrane injury and loss of surface cells.

An ultrastructural examination of rat corneal epithelium following repeated topical applications of 0.4% and 1.0% benoximate, 4.0% cocaine, 2.0% lidocaine, and 0.5% tetracaine was reported by Leuenberger (1973). With each of the anesthetics, scanning and transmission electron microscopy revealed a loss of microplicae and microvilli on the corneal surface, corresponding to a fine corneal stippling observed with the use of a slit lamp. No basic differences in the type of ultrastructural changes or severity of lesions were found for the different anesthetics.

Overall, these results suggest that further research is needed to adequately demonstrate the acceptability of an anesthetic for use in eye irritation testing. At present, tetracaine appears most promising.

3.8 OBSERVATION PERIOD

The original Draize test involved grading reactions at intervals of 24 hours to 7 days or longer for persistent injuries. Observation at 24, 48, and 72 hours is required by the FHSA and has been proposed by the IRLG. The OECD guidelines recommend observation at 1, 24, 48, and 72 hours. While the FHSA method does not specify the use of extended observation periods, the IRLG states that the eyes may also be examined at 7, 14, and 21 days, at the option of the investigator. The OECD recommends an extended observation period to determine the progress and persistence of the lesions, but observation normally need not exceed 21 days after instillation. The NAS recommends an observation period of 1 to 21 days. The following public comment was offered in response to the proposed IRLG guidelines: "Extended observation (at least three weeks) with emphasis on rate of recoveries of lesions rather than on their interim intensity must be emphasized."

According to Green et al. (1978), whether a material has caused structural change suggestive of permanent impairment of vision cannot be determined until enough time has elapsed to permit healing. Based on the results of their investigation of responses in the rabbit and monkey, they concluded that (1) the observation period must extend up to three weeks for adequate evaluation of the effects produced by introducing chemicals into the eyes of animals, (2) some injuries may not be evident until after three days have passed and many lesions undergo significant healing within 21 days; and (3) it is not possible to predict the ultimate outcome of the injury using observations made only during the first three days after instillation of the chemical.

Guillot et al. (1981) found that, for 16 of 56 chemicals tested for eye irritation in rabbits, reactions observed up to 72 hours after instillation were less severe than those observed after an observation period of 7 days. Both methods were adequate, however, in differentiating a positive from a negative response in all cases.

A 72-hour observation period following instillation appears adequate to define the irritation potential of a test material. Extended observation periods increase the capacity of the test to detect delayed reactions and to characterize the reversibility of lesions. Observation periods of up to three weeks, therefore, are suggested when fuller characterization of the irritation response is required.

4.0 ASSESSMENT OF THE IRRITATION REACTION

Key variables in the complex process of assessing the results of eye irritation tests are the techniques used to examine the eye, the types of responses that are recorded, the scoring system used, and the evaluation and interpretation of the lesions produced.

4.1 EXAMINATION TECHNIQUES

Assessment of the ocular reaction can be facilitated by the use of a slit lamp. Slit lamp biomicroscopy allows for accurate differentiation of clinical signs in the transparent structures of the eye (cornea, aqueous humor, lens, and vitreous body) by providing observation in optical section with the slit beam and the microscope (Gelatt, 1981; Aronson, 1975). All slit lamp biomicroscopes have two basic components: an illuminating system of adjustable slit width and a binocular microscope for magnification. The light can be moved freely and directed at the eye in the frontal plane as well as at various angles to the eye and the biomicroscope. The table mounted slit lamp biomicroscope offers maximum flexibility in illumination and magnification (up to 40x) as well as photographic capability. The portable hand held model possesses slit widths of generally 0 to 10 millimeters and magnification of 5 to 20x. Examination of the location and extent of ocular lesions and the measurement of corneal thickness are accomplished with the slit lamp biomicroscope. Green et al. (1978) have found slit lamp examination to be more sensitive than gross external observation for detecting long-lasting damage to the eye. Baldwin et al. (1973) have described the use of photography, using a photoslit lamp, in documenting ocular pathological changes. Eye irritation reactions have also been examined with the use of an ophthalmoscope (Weltman et al., 1965; Guillot et al., 1981).

Fluorescein staining is also a valuable aid in defining corneal epithelial damage. Fluorescein allows visualization of very small superficial lesions of the cornea which might otherwise be completely overlooked. When the superficial layers of the epithelium are damaged, the dye is readily taken up by the remaining deeper layers and it will fluoresce when ultraviolet light is cast on the area (NAS, 1977). Interpretation is facilitated by rinsing the eye with an irrigating solution to remove excess and nonabsorbed fluorescein.

Fluorescein dye is available in two commercial forms that are suitable for ocular testing: sterile ophthalmological solutions containing 0.25% to 1.0% sodium fluorescein and individual hermetically sealed paper strips containing fluorescein. In response to the proposed IRLG guidelines, one commenter felt that the possibility of contamination of the fluorescein solutions was a concern. The commenter suggested that contamination could be circumvented by using the fluorescein strips.

4.2 SUBJECTIVE SCORING

Perhaps the greatest difficulty with eye irritation testing is the subjective scoring and evaluation of corneal damage. The original Draize test ranked the total ocular irritancy of test materials on a scale of 0 to 110. This score was based on the total of the individual subjective scores for the cornea, iris, and conjunctiva, each multiplied by a factor (Appendix A). The resultant weighting or "biasing" of the total score was designed to compensate for the generally perceived importance of each graded tissue in the normal visual process. Serious lesions such as pannus, phlyctena, and rupture of the eyeball are also reported. The methods established by FHSA and recommended by the IRLG attribute greater significance to the individual scores obtained in the examined ocular tissues. These two methods and the method proposed by the OECD all utilize the same system for grading ocular lesions. This system, as described in the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances" (CPSC, 1976), is presented in Appendix B.

In the FHSA and IRLG protocols, a minimum of one positive response observed in any of the examined tissues of at least four rabbits indicates a positive test result. If only one animal exhibits a positive reaction, the test result is regarded as negative. When two or three animals exhibit a positive reaction, the IRLG guidelines provide for either designating the substance as an irritant or, as is required with the FHSA method, the test is repeated using a different group of six animals. The second test is considered positive if three or more animals exhibit a positive reaction. The FHSA method further requires a third test if only one or two animals in the second test exhibit a positive reaction. If any animal in the third test exhibits a positive reaction, the substance is regarded as an irritant. The FHSA and IRLG methods differentiate an irritant from a non-irritant but do not rank substances according to the degree of irritancy which they produce.

According to the OECD proposed method, the individual scores for ocular reactions do not represent absolute scores for the irritant properties of a material. Rather, they should be viewed as reference values and are meaningful only when supported by a full description and evaluation of the observations.

For scoring of ocular irritant effects, the National Research Council of the National Academy of Sciences (NAS, 1977) proposes both the original scoring scheme of Draize and a technique using a slit lamp. With the latter technique, the total individual score obtained at any one observation period is the sum of all the ratings for the cornea, iris, lid, and conjunctiva. The NAS also recommends including persistence of response as a criterion in the classification of test substances in terms of irritancy.

Kay and Calandra (1962) developed a system for rating eye irritants based on the Draize scoring method. The extent and persistence of irritation and the overall consistency of the data form the basic criteria for the assignment of ratings from "non-irritating" to "maximally irritating." Guillot et al. (1981) used a similar system for the interpretation of test results. A mean index of ocular irritation (Draize scoring system) is obtained for six treated rabbits at each observation time. The highest mean index of ocular irritation obtained is used to sub-classify the test substance between the limits of "non-irritant" to "extremely irritant." A final rating is then assigned based on the rate of recovery in a specified number of animals.

Ballantyne and Swanston (1977) have discussed the methods of scoring and grading eye effects. They cited three major problems with the Draize scoring system. The system describes only selected effects on three tissues and as a result may yield an incomplete picture of the reaction of an eye to a given chemical. Marked effects on tissues considered less important are de-emphasized by the grading system which can lead to difficulties in interpretation. Thirdly, the results are reported not as the grades of effects seen but as biased scores arithmetically derived from the grades observed. The scoring system specified by the FHSA was criticized for specifying effects on only three tissues and for the resulting uneven distribution of the scores for the various effects observed. The scoring system of Ballantyne et al. (1974) was cited as an attempt to obtain a more uniform approach to grading. This system records the scores for lacrimation, blepharitis, chemosis, injection of conjunctival blood vessels, iritis, keratitis, and vascularization of the cornea, each on a six point scale.

Another criticism of the Draize scoring system has been its inability to correlate active inflammatory signs with permanent structural change. Aronson (1975) has described a technique for observing parameters in animals identical to those observed in human studies and which introduces a classification of ocular structural change occurring as the result of active inflammation. All observations are performed with a slit lamp. The degree of inflammation required to produce structural change is determined for the cornea. For example, a corneal infiltrate greater than 2.0 millimeters in diameter must be present for approximately four days before neovascularization of the cornea occurs. The author notes that examination using a number of ocular irritants would be necessary to establish whether the relationship between active inflammation and structural change was statistically reproducible.

Interlaboratory variation in eye irritation scores also has been studied. In 1962, Russell and Hoch reported rabbit eye irritation results of two variations of a shampoo formula tested by five different laboratories. Good agreement was observed between scores for rabbits given identical treatment within the same laboratory, but differences in scores and the resulting irritancy classifications of the tested samples were found between laboratories. The authors recommended that procedures be standardized and that a standard substance be developed which could be used by any testing laboratory to determine whether it is scoring at a "standard" level.

In a similar study, seven liquid materials were evaluated in the Draize rabbit eye irritation test by 10 laboratories (Marzulli and Ruggles, 1973). Each laboratory was furnished with the test methodology and eye irritation score sheets to be used. Each substance was tested in six albino rabbits of either sex. A volume of 0.1 milliliter of the test material was instilled onto the cornea of one eye of each animal and four parameters (corneal opacity, iritis, conjunctival redness and chemosis) were evaluated at 24, 48, and 72 hours and 7 days. Inclusion of all four criteria for eye irritation resulted in a high degree of consistency among evaluators in separating an irritant from a non-irritant. However, the test was inadequate if only a single parameter was used to make the evaluation. Marzulli and Ruggles concluded that the Draize test, as a first step, is capable of providing reliable and reproducible information regarding the eye irritant properties of test materials.

Weil and Scala (1971) in a collaborative study documented substantial variation in ocular scores within and among 24 laboratories (nine consultant; six food or industrial chemical; seven toilet goods and cosmetics; and two governmental laboratories). Reference procedures similar to the Draize method and reference materials were supplied to each of the participating laboratories. Certain materials that were rated by some laboratories as the most irritating among those tested were rated as the least irritating by others. Weil and Scala concluded that in most cases the variation between laboratories resulted primarily from the reading of the reaction as opposed to variation in interpretation and performance of the procedures, although the latter factor was also considered a component of the interlaboratory variability. The authors commented that "unconscious bias or definite tendencies to over or underread reactions or misinterpret the meaning of descriptive terms can be counteracted only by having several or many laboratories perform similar tests."

A statistically based experimental design has been devised by McDonald and Shadduck (1977) to examine variability in subjective scoring among a group of investigators (analyst uniformity) and within the same investigator (analyst precision). The design provides for four groups of rabbits consisting of 24 eyes per group. Test groups receive topical ocular treatments that result in reactions ranging from non-irritating to severely irritating. A first group of 48 eyes is tested in the morning and a second group in the afternoon. Observation of each group is confined to one hour in order to minimize the effect of time on ocular reactions. To assess analyst precision, each eye is observed twice without prior knowledge of treatment. The degree of reproducibility is evidenced by the tabulated scores for each ocular parameter. For example, a conjunctival swelling score of two was given on 12 occasions in both trials by the same investigator, who on seven other occasions gave a score of three in the first trial and a score of two in the second trial for a given treatment regimen. The precision of each of three analysts was found to range from 73% to 88% for conjunctival congestion. A reproducibility of generally 80% or better was observed for swelling, light reflex, corneal intensity, iritis, and flare. This was considered to be good precision, for a subjective scoring system. For discharge, percentages ranged from 57-79 which was considered inadequate. Analyst uniformity for three investigators was considered to be very good for all ocular parameters except congestion and discharge. Correlation coefficients were 0.90, 0.97, and 0.92 for corneal intensity and 0.63, 0.59, and 0.61 for congestion for investigator A vs B, A vs C, and C vs. B, respectively. The authors conclude that this experimental procedure provides a sufficient number of eyes for statistical evaluation and is an effective means of monitoring and improving investigator reproducibility. Related tests to assess individual reader consistency (Bayard and Hehir, 1976) showed that each of three readers reproduced a positive or negative score more than 90% of the time.

Historically, scoring systems based on subjective examination have been universally used and generally are considered adequate; although, as shown by the above studies, the use of these systems has led to inaccuracies in the assessment of eye irritants. Without careful standardization of the details of the test method as well as the observation and scoring system, significant interlaboratory variation is possible. With proper controls, subjective scoring systems generally produce valid and useful information in a screening test and, with the use of simple aids such as a standardized pictorial guide for scoring lesions (see Appendix B), their accuracy can be measurably improved. Total evaluation of each score obtained at each observation period is necessary. Substances which are likely to come in contact with the human eye due to anticipated patterns of production and use may require further evaluation by more sophisticated methods.

4.3 OBJECTIVE METHODS OF ASSESSMENT

To overcome the disparities that can result from the subjective scoring of eye irritation reactions, objective techniques have been combined with and compared to the Draize examination procedure. These techniques have included the measurements of corneal thickness, intraocular pressure, corneal and conjunctival weight, and capillary permeability, as well as performance of histological analyses. Objective methods of assessment may be useful as aids to facilitate the evaluation, and they have the advantage of being more readily standardized.

4.3.1 Corneal Thickness

Burton (1972) described the use of corneal thickness measurements for assessing the corneal irritation produced in rabbits by materials of differing degrees of irritancy. Corneal thickness was measured a few hours prior to instillation and thereafter at intervals of 24, 48, 72, and 96 hours. For comparison, reactions were also scored by the Draize method. Corneal swelling was determined by dividing the corneal thickness value obtained on the day of observation by a value obtained prior to treatment. This proportionality assumes that changes in refractive index and radius of curvature (both of which influence corneal thickness measurements) are a function of the degree of swelling which at least partially compensates for individual variations in thickness of the untreated cornea. Burton noted that, since the cornea swells with a high degree of uniformity, measurement of corneal thickness at the corneal apex alone is sufficient. Photographs of treated eyes show that the thickness of the slit image is an approximate measure of the thickness of the cornea; greater swelling is associated with greater irritation as assessed by the Draize method; and measurements of slit image thickness can distinguish subtle differences between reactions given the same subjective score.

In the Burton study, corneal swelling observed in 100 rabbits treated with a variety of materials was compared with the Draize scores recorded on the same day. Corneal swelling correlated closely with both the Draize opacity score and total corneal score. A relationship between conjunctival reaction assessed subjectively and the degree of corneal swelling also was observed, but the correlation was not as good as that for the total corneal score. To investigate the relationship between the persistence of reaction and corneal swelling, an analysis was made of the results of experiments on 100 rabbit eyes to which 34 different materials had been applied and in which swelling in excess of 130% of the untreated thickness had been noted. The maximum corneal thickness recorded during the first four days of observation was used to express corneal swelling. Subjective assessment (without a slit lamp) classified reactions as persistent in 57 of the 100 rabbit eyes observed. The mean corneal swelling of this group was 164.14% ± 18.08%. The remaining 43 were judged not to have reacted persistently. These eyes had corneas swollen to a mean extent of 156.72% ± 14.96% ($P < 0.05$ compared with the value for substances classified as causing persistent reactions). Persistent corneal damage was, therefore, associated with greater corneal swelling. Burton concluded that the corneal thickness method of assessing corneal damage would be effective in minimizing laboratory discrepancies if the method of measurement and possibly the age/size range of the rabbits used were standardized to some degree.

Draize scores in relation to objective assessments of ocular irritation, including measurement of corneal thickness, were also investigated by Conquet et al. (1977). Seven organic solvents ranging from non-irritating to very irritating were tested in rabbits. Two hours after instillation of the test solvent, the Draize score did not reveal any corneal changes, although significant corneal thickness variations compared with those of the controls were found for four of the seven compounds tested ($P \leq 0.05$). At 24 hours, both methods detected corneal changes, but for two of the solvents (dimethylsulfoxide and Carbitol®) corneal thickness measurements differed from control measurements ($P \leq 0.05$), whereas the Draize corneal scores did not differ from control scores. Both corneal thickness measurements and Draize total scores, however, identified the same compounds as irritants. Gradations of irritancy were similarly assessed by the two methods at two hours (correlation coefficient, $r = +0.86$) and 24 hours ($r = +0.99$). There was no significant correlation between the two methods at days 3, 7, and 11 ($r = +0.71$, $+0.48$ and $+0.72$, respectively). The authors conclude that the Draize procedure did not permit adequate detection of increased corneal thickness and, therefore, suggest that specific corneal thickness measurements be made in addition to the standard Draize test.

In rabbit eye tests with a 10% solution of sodium lauryl sulfate, a maximum response in corneal thickness was observed at two hours (0.50 ±0.04 millimeters compared to baseline value of 0.34 ±0.08), whereas a maximum response in clinical parameters (redness, swelling, discharge) was observed at four hours (Walton and Heywood, 1978). Burton et al. (1981) used corneal thickness measurements to assess the irritancy of chemicals applied to enucleated rabbit eyes. The *in vitro* measurements, expressed as corneal swelling, showed a generally good agreement with published results of eye irritation in rabbits.

The above studies and a review by Heywood and James (1978) indicate that the measurement of corneal thickness may be a valuable supplement to the standard eye irritation scoring system. Measurements of corneal thickness will increase the time and expense of testing but they also provide the advantage of using the same eye as its own control.

4.3.2 Intraocular Pressure

The technique of applanation tonometry has been used to measure intraocular pressure in the assessment of eye irritation. This technique measures the force required to produce a degree of flattening of the cornea (Heywood and James, 1978).

Walton and Heywood (1978) performed a subjective clinical assessment, corneal thickness measurements, and applanation tonometry throughout a 24-hour period on rabbit eyes treated with a 10% solution of sodium lauryl sulfate. A calibrated Perkins hand-held tonometer (applanation) was used to measure intraocular pressure. Prior to this measurement, the cornea was anesthetized with one drop of 0.5% proparacaine hydrochloride. A small drop of evaporated milk was applied to the tonometer prism to visualize the applanation rings. The eyes were thoroughly irrigated with normal saline after each of three tonometer readings.

The intraocular pressure increased more quickly and returned to near normal sooner than the other factors measured. The highest value was reached at 30 minutes after instillation of the irritant (32.17 ± 2.11 millimeters Hg; $P < 0.001$ compared to baseline value of 19.33 ± 1.47). At 24 hours, intraocular pressure remained elevated above baseline ($P < 0.05$). The untreated eyes also experienced a rise in intraocular pressure ($P < 0.05$ at 2 hours). Increases in corneal thickness could be correlated to increases in intraocular pressure. While swelling persisted throughout the observation period, corneal thickness showed maximal response after two hours. The maximal response in clinical parameters was observed after four hours, at which time the corneas were dull and there was evidence of iritis. Walton and Heywood (1978) conclude that measurement of intraocular pressure may be of value in the assessment of local eye irritation but it significantly increases the number of variables associated with the assessment. This measurement methodology requires local anesthesia, introduction of a second material in order to visualize the applanation rings, and thorough irrigation following the procedure, all of which could influence the long-term effects of the primary irritant.

Ballantyne et al. (1972) investigated the effects of different concentrations of various materials on intraocular tension in the rabbit. Measurements were made with a hand-held applanation tonometer. Ammonia, butyl carbitol, ω-chloracetophenone and o-chlorobenzylidene malononitrile all caused significant dose response increases in intraocular tension at 10 minutes. Tensions returned to near normal by 1 hour. With ammonia and ω-chloracetophenone, higher concentrations produced marked increases in the rise of intraocular tension per incremental increases in concentration. With butyl carbitol and o-chlorobenzylidene malononitrile, the rise in tension per increase in concentration was relatively uniform over the range studied. While there was no evidence that these changes were a measure of irritancy, the authors suggest that a relationship may exist between the rise in tension and the severity of the subsequent reaction.

Intraocular pressure measurements were included in an objective evaluation of the ocular irritation produced by topically administered nitrogen mustard in rabbits (Maul and Sears, 1976). Nitrogen mustard elicited dose/response increases in intraocular tension. Investigators in an earlier study utilizing applanation tonometry (Smith and Mickatavage, 1963) felt that intraocular pressure measurements as ordinarily determined in such animals as rabbits, dogs, and cats were not reliable. Such measurements, therefore, were limited to human test subjects as an additional correlation with local toxicity responses. The use of intraocular pressure measurements in the assessment of responses in eye irritation testing requires further study.

4.3.3 Corneal and Conjunctival Edema

In tests with seven organic solvents, Conquet et al. (1977) measured the corneal and conjunctival water content of treated rabbit eyes. Animals were killed at 2 and 24 hours following instillation of the test agent. At two hours, corneal edema (percent dry weight/wet weight) was different from controls for five of the compounds ($P \leq 0.05$). Draize corneal scores, however, indicated no reaction with any solvent at the two-hour observation period. At 24 hours, the degree of corneal edema produced by three solvents (different from control; $P \leq 0.05$) corresponded with a positive reaction assessed by the Draize corneal score. A significant linear correlation was also found between corneal thickness and corneal edema measurements at both 2 hours ($r = +0.95$) and 24 hours ($r = +0.93$). Conjunctival changes were detected by edema measurement and the Draize procedure. A significant correlation was found between both methods at 2 hours ($r = +0.94$) and 24 hours ($r = +0.96$).

Lallier et al. (1975) determined that corneal and conjunctival weights (edema) are precise and repeatable measurements. Based on results with organic solvents, these measurements were suggested to be of use for the quantitative assessment of ocular irritation. The data reported by Lallier et al. (1975), however, show that the percentage dry weight (cornea) never fell more than 10% below the control value with even the most severe irritants (Heywood and James, 1978). Heywood and James (1978) also cited the findings of Wright et al. (1976) in which the water content of the cornea showed no direct relationship to opacity. The value of corneal and conjunctival edema measurements in eye irritation testing is not readily apparent.

4.3.4 Capillary Permeability

Changes in capillary permeability have been assessed by determining the concentration of dye per gram of dry weight of conjunctiva or per milliliter of aqueous humor following intravenous injection (Conquet et al., 1977). Following instillation of various test solvents, a significant linear correlation was found between capillary permeability in the conjunctiva and the Draize conjunctival score at 2 hours ($r = +0.96$) and at 24 hours ($r = +0.98$).

While Lallier et al. (1975) reported that the increase in the capillary permeability at the blood/aqueous humor level appears to be the first event occurring in eye irritation, Conquet et al. (1977) found that the diffusion of dye in the aqueous humor did not allow a comparison with scores achieved by other methods, particularly the Draize iris score. Maul and Sears (1976) observed increases in the amount of intravenously injected dye recovered from the conjunctiva and iris of rabbit eyes following treatment with nitrogen mustard.

4.3.5 Histological Investigation

Histological evaluation of ocular lesions entails the description and classification of lesions by a pathologist and is, therefore, a subjective assessment. However, the lesions are examined at the microscopic level and are systematically recorded in considerably greater detail than with a macroscopic subjective assessment. The information obtained on structural change provides a more complete evaluation of ocular damage. In practice, the histological evaluation of eye damage is sufficiently sensitive and reliable for this technique to be included with the objective methods.

Heywood and James (1978) summarize the parameters which should be evaluated in a histological examination of eye irritation: "The normal cornea should show microvillae in the superficial cells of the corneal epithelium, microtubules in Descemet's membrane and vesicles in the endothelium, indicating active transport systems. There should be no oedema and the collagen fibres in the stroma should be of normal size and show no distortion. In the conjunctiva, there should be normal microvillae and the epithelium cells should be rich in endoplasmic reticulum. Normal goblet cells are of considerable importance, as they are responsible for the mucin in the precorneal film. Alterations in any of these parameters must be considered of significance."

Histological observation was included by Weltman et al. (1965) in a comparative evaluation of techniques used to assess eye irritation. In rabbit eye tests with two shampoos, a correspondence was noted between the eye irritation scores determined at the time of death and the degree of histologic damage. Approximately two-thirds of the treated eyes showed considerable erosion in the stratified squamous layers of the cornea during the first three days following instillation of the test preparations. By the seventh day, all the eyes treated by both substances were histologically normal in appearance, consistent with the evaluation by the Draize procedure.

Tonjum (1975) studied the effects of benzalkonium chloride on the rabbit corneal epithelium. Within two minutes of application of the irritant, the superficial cells displayed small holes and a loss of microvillae. Histological analysis was performed by Mel'nikova and Rodionov (1979) in determining the ocular pathology in the rabbit and guinea pig eye produced by the antibiotic grisein.

Histological analysis was also used to classify the responses of the rabbit and monkey eye to a variety of test substances (Green et al., 1978). A discrepancy was found, however, between the results of histopathological and slit lamp examinations. At 21 days following treatment, histopathologic analysis detected a few lesions which were not detected by the slit lamp. These findings were considered consistent with the supposition that pathologic examination is the more sensitive method. In several cases, however, histopathologic examination failed to detect changes that had been judged by the slit lamp to be evidence of injury. This was attributed to a possible failure to section the eye through an area containing a small lesion, though differences in interpretation of the evidence presented by the two methods were also thought to be a possible factor in the discrepancy.

4.3.6 Photographic Observation

An investigation of ocular irritation by Weltman et al. (1965) included the use of photography. Immediately before autopsy of rabbits scheduled for histological analysis of the ocular tissues, the eyes were photographed twice. The first picture was taken with the treated eye at rest and the second one with the upper and lower lids held apart. Histologic observations were made from different sections of each eye. Examination of the photographs revealed that the external, physical appearance of the test and control eyes corresponded to the conditions shown by the various histologic sections.

Photographs of slit images obtained from untreated and treated rabbit corneas are included in a report by Burton (1972). The "Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances" (CPSC, 1976) contains photographs of rabbit eye lesions which correspond to the various grades of irritancy. Baldwin et al. (1973) have suggested the use of photographic recordings of ocular lesions for instructional purposes. Photographs and descriptions of reactions in the rabbit and monkey eye included in a report by Green et al. (1978) were considered by the authors to contribute a substantial data base for comparing other substances with those employed in their investigation.

4.3.7 Additional Techniques

In an objective evaluation of the ocular irritation produced by nitrogen mustard in the rabbit eye, Maul and Sears (1976) examined changes in pupil diameter and the level of protein in the aqueous humor. The latter changes were determined by refractometry of samples obtained through corneal paracentesis. Miosis occurred in the pupil within minutes after application of the irritant at concentrations of 0.1% to 10%. The irritant also elicited dose/response increases in protein concentration in aqueous humor ($P < 0.01$ at 0.1% nitrogen mustard compared with control). These results, together with measurements of intraocular pressure and hyperemia of the conjunctiva and iris, allowed an analysis of the possible mechanism and pathway of nitrogen mustard-induced ocular irritation in rabbits. The results support the hypothesis that the effects in the eye are produced by a specific transmitter/receptor mechanism for one or more substances released by pain fibers in response to the induced irritation. Pupillometry was also studied by Krejci and Krejcova (1974). Rabbit eyes (and cultures of rabbit corneal epithelium) were treated with N-butyl gallate and epinephrine. Changes in pupil diameter were compared with development of degenerative changes in tissue culture.

Heywood and James (1978) reported that measurements of corneal curvature are precluded because of inaccuracies that result from the disturbing of the precorneal film and the superficial epithelial cells of the cornea by irritants. The technique of enzyme histochemistry has been used by Gasset et al. (1974) to compare the effects of three ophthalmic preservatives on rabbit eyes. NADH2-oxidoreductase staining was used to test the viability of the endothelium. Szary (1977) combined slit lamp observations and aqueous humor measurements of protein, sialic acid, seromucoid, and proteolytic activity to evaluate the intensity of inflammatory changes in the anterior chamber of the rabbit eye. Increased ocular inflammatory changes have been correlated with increased ocular temperatures measured with an infrared thermometer (Ashford and Lamble, 1974).

Specular microscopy has been used to visualize the endothelial and epithelial layers of corneas both *in vivo* and *in vitro*. The use of clinical specular microscopy in ophthalmological studies has been reviewed by Sugar (1979). Scanning and transmission electron microscopy may prove useful for evaluating cellular toxicity of topically instilled products (McDonald and Shadduck, 1977). The major drawback of electron microscopy in the evaluation of ocular tissue damage is the requirement for observation of chemically fixed tissues in the dehydrated state (Burstein, 1980). Cryofixation has been described as an alternative to chemical fixation of surface tissues (rabbit corneal endothelium) for viewing by electron microscopy (Burstein and Maurice, 1978).

5.0 ADDITIONAL *IN VIVO* STUDIES

5.1 TESTS IN HUMANS

Studies in human volunteers are valuable for predicting the irritancy of products used in and around the eyes. These studies typically follow the eye irritation test in animals and provide a direct evaluation of a particular product's safety.

A one-day method was devised by Callahan et al. (1979) for studying acute ocular irritation effects in humans from an over-the-counter ophthalmic preparation. Sixty-one subjects of either sex participated in the study. Prior to dosing, the eyes of each subject were examined with a slit lamp and given a baseline rating. One eye received two drops of the active solution (containing 0.05% tetrahydrozoline) and the other eye received two drops of a placebo (the same vehicle but without tetrahydrozoline). This procedure was repeated every three hours until four doses had been administered. Five minutes after each dosing, the investigator's clinical findings, as well as the participants' subjective assessments of burning, stinging, or itching, were given numerical ratings relative to the degree of severity. Neither the investigator nor the subject knew which eye had the active solution. The clinical parameters investigated were lacrimation, palpebral and bulbar conjunctival hyperemia, and lid edema. The test product proved to be non-irritating. The majority of clinical scores achieved during the testing period were for mild palpebral and conjunctival hyperemia. As would be expected from the vasoconstrictive properties of tetrahydrozoline, however, fewer of the eyes which received the active solution containing tetrahydrozoline showed conjunctival hyperemia. No cumulative dose/response effect was apparent; subjects with positive clinical scores or subjective symptoms from either solution were fairly evenly distributed throughout the four evaluation periods. Since only a few reactions were observed, an additional five-day irritancy protocol was not initiated. The authors concluded that a carefully monitored safety study is necessary for detection of subtle changes that may constitute or lead to unforeseen ocular damage in the user of an ophthalmic preparation.

Van Abbe (1973) reported that both rabbit eye tests and human usage trials failed to predict eye irritation under certain product use conditions. A hairdressing for men, which was marketed after initial screening tests indicated that it was safe, was reported to be an eye irritant in 1 in 8,500 purchases. In every instance, the complainant had been exposed to rain or snow after using the product. In some of these cases, ophthalmologists noted pitting of the corneal epithelium and a positive response to fluorescein. Reevaluation showed that rabbit and monkey eye tests were negative for both the diluted and undiluted product. However, definite irritation was observed when rinsings (simulating exposure to rain or snow) taken from the human head after normal use of the hairdressing were concentrated by freeze-drying and instilled into rabbit eyes. Transient corneal wrinkling or pitting was noticeable one to two hours after initial instillation and became more prominent after a second instillation. Corneal pitting was not seen after 24 hours. When single ingredients of the product were tested in aqueous solution, only one ingredient, 13-mole ethoxylated oleyl alcohol, gave a definite irritant response. Transient corneal wrinkling or pitting at one to two hours and a slight reddening of the conjunctiva at 24 and 48 hours were observed. Much greater irritancy was shown on animal skin than in the eye. When 0.01 milliliter of the product was applied daily for four days in concentrated form or diluted up to 1:4 with water, irritation occurred in intact and abraded rabbit skin and on the ears of female CF/1 mice. The same product did not have an effect on human skin in patch testing or in normal use.

In experiments designed to corroborate the animal findings, human volunteers used the product daily for five days. No irritation was reported after rinsings from the head were instilled in one eye of the user four times daily for five days. Although aqueous solutions of the 13-mole ethoxylated oleyl alcohol did produce a burning sensation, lacrimation, and erythema, the delayed response reported in complaints from users was not seen. Van Abbe suggests that, in the application of rabbit eye test results to humans, investigators should perhaps pay more attention to corneal pitting or wrinkling. A second instillation of test agent may also be necessary, since in this case a positive response was obtained in the rabbit eye only after two instillations.

Schuck et al. (1966) devised a method for measuring the eye irritancy produced in humans by photo-oxidation products of polluted air. Conditions of photochemical air pollution were simulated in a large reaction chamber equipped with light sources capable of generating an intensity equivalent to noonday sunlight. The eyes of the subjects were exposed to the chamber atmosphere via welding masks mounted in the sides of the chamber. Subjects indicated the intensity of irritation by adjusting a scalepointer throughout a five-minute exposure period and an investigator recorded their rate of blinking. A set of 70 experiments was conducted in which formaldehyde was produced in the parts per million range by irradiation of ethylene. A linear relationship was found between reported eye irritation and formaldenyde concentrations greater than 0.3 parts per million. A reverse relationship was observed for lesser concentrations. Most subjects experienced the same irritation intensity at concentrations of 0.05 parts per million of formaldehyde as they did at 0.5 parts per million. In some subjects the relationship between blink rate and reported severity of irritation appeared to be inverse. In similar experiments, peroxyacyl nitrates were produced by the irradiation of propylene. The authors suggest that eye irritation produced by photochemical air pollution can be predicted by the level of formaldehyde and peroxyacetyl nitrate.

5.2 COMPLEMENTARY TESTS

Irritation profiles that assess sensory irritation (sting/burn) in addition to primary irritation may be useful in determining the ocular hazards posed by substances as well as the acceptability of commercial products to consumers. Tests which have been used to evaluate sensory irritation include the blepharospasm (closing of the lids) test, the mouse writhing test, and the mouse upper respiratory tract test.

The concentration of the riot control agent o-chlorobenzylidene malononitrile (CS) required to produce a blepharospasm response in 50% of a group of test subjects (EC_{50}) was compared in guinea pig, rabbit, and man (Ballantyne and Swanston, 1973). The human eye was about seven times more sensitive to this irritant than the guinea pig eye and about 18 times more sensitive than the rabbit eye. In a similar study, Ballantyne and Swanston (1974) also reported species differences in blepharospasm response to dilute solutions of dibenzoxazepine. The human eye was demonstrated to be 40 times more sensitive than the guinea pig eye and 90 times more sensitive than the rabbit eye. Because of the species variation, the investigators concluded that results from animal threshold studies could not readily be extrapolated to man.

In view of the inadequacy of the Draize test for predicting discomfort or stinging in human eyes, Shanahan and Ward (1975) investigated the mouse writhing test as an animal model for estimating the relative sting potential of eight experimental and three commercial shampoos. Male mice were injected intraperitoneally with 0.2 milliliter of graded shampoo concentrations in water. The writhing response was characterized by contraction of the abdominal musculature and was often accompanied by tension and flexion of the trunk and extension of one or both of the hind limbs. One writhe per mouse was considered a positive response as were other overt symptoms indicative of pain or discomfort. Results were compared with the sting response from test material dilutions in human eyes and with the irritation produced by full strength concentrations in the Draize rabbit eye test. The mouse writhing test successfully identified four shampoos which were significantly more irritating than the reference shampoo. Tests in humans also revealed that these four shampoos produced a high degree of eye sting. The mouse writhing test also determined that six of the shampoos were equivalent in mildness to the reference shampoo. Sting evaluations in human eyes similarly showed these six to be the mildest. Only one of the shampoos produced significant, visible irritation in the Draize rabbit eye test. Shanahan and Ward suggested that the mouse writhing test would be a valuable adjunct in predicting the potential for ocular discomfort and stinging.

Dossou and Pamart (1979) also used the mouse writhing test to determine the sting capacity of shampoos. Serial dilutions of each of 22 shampoos were investigated following intraperitoneal injection in male and female Swiss mice. The number of mice showing a reaction and the DC_{50} (concentration necessary to produce writhing in 50% of a test population) were determined for each product. The baby shampoos generally were more mild than the adult shampoos. The mouse writhing test was also performed on selected primary constituents of the shampoo formulas. DC_{50} measurements were higher for male mice than for females. The sting capacity appeared to coincide with the irritancy of the ingredients as determined by the rabbit eye test. In a related study of 13 shampoos, however, no correlation was apparent between Draize scores and the DC50 values found in the mouse writhing tests.

Alarie (1966) investigated the sensory irritant potential of various substances using the mouse upper respiratory tract test. This test determines irritancy by monitoring the respiration rate in restrained mice exposed to aerosolized materials. Low exposure concentrations are used to determine sensory irritation without cellular damage. Irritation was found to correspond quantitatively and reproducibly with a decrease in respiratory frequency and an increase in the duration of expiration. The sensory irritant effects of 52 compounds were tested in humans and mice. After humans were exposed to the test agents, responses were evaluated in terms of eye, throat, skin, nose, or chest burning; conjunctivitis; lacrimation; coughing; and gagging. Compounds found to be either non-irritating or irritating in humans were similarly classified by the mouse upper respiratory tract test. Thus, such tests in mice appear to permit a qualitative extrapolation to humans of the sensory irritant potential of materials in the human eye.

The irritancy levels of several widely used surfactants were ranked comparatively in animal test determinations of sensory irritation, pain response, and tissue damage (Ciuchta and Dodd, 1978). Rabbit blepharosphasm and mouse writhing tests were performed, as were the standard Draize rabbit eye and skin irritancy tests. In addition, the sensory irritation potential of the surfactants was objectively evaluated by the mouse upper respiratory tract test. For this test, respiration rates were determined in chambered mice exposed to aerosolized 15% solutions of the test surfactants. Both the mouse writhing test and upper respiratory tract test permitted classification of the test materials in terms of sensory irritation potential. Because the surfactants induced ocular anesthesia, the blepharospasm test did not lend itself to sensory evaluation of the test agents. Almost all of the surfactants received basically the same scores in the rabbit eye irritancy tests. The rabbit skin irritancy test was more effective in differentiating the irritancy of test agents, though pattern shifts were noted at different concentrations.

The ocular anesthetic effect of certain products has been investigated further in humans and rabbits. Prompted by the possibility that users of soaps and surfactants capable of causing corneal anesthesia may unknowingly damage their eyes, Harris et al. (1975) studied the anesthetic effect on human and rabbit corneas of five common bath and facial soaps and four commercial shampoos. Shampoos were tested at full concentration and at dilutions of 1:2 and 1:4. Soaps were tested as 10% solutions. For purposes of comparison, one group of rabbits and one group of humans received 0.5% tetracaine. The degree of corneal anesthesia was measured with an esthesiometer, consisting of a nylon filament of controllable length with numbers that correspond to the pressure exerted on the cornea. Anesthesia was assessed by the numerical value at which a blink reflex was elicited. The mean difference in esthesiometer readings between treated and untreated eyes at each time interval was analyzed. All of the test compounds produced anesthesia in rabbits that was similar to that produced by tetracaine, but much longer lasting. Tetracaine-induced anesthesia lasted between 20 and 30 minutes in human subjects and was comparable with that seen in rabbits. None of the soaps or shampoos, however, produced corneal anesthesia in humans. The results indicated that findings of corneal anesthesia induced by surfactants in rabbits may not be extrapolated to the human eye.

While results of the mouse writhing test (Shanahan and Ward, 1975) and the mouse upper respiratory tract test (Alarie, 1966) correlate with the human sensory response to irritants, Heywood and James (1978) stated that animal systems cannot be used to measure the phenomenon of stinging in humans. According to Buehler (1974), methods are needed for measuring both pain and anesthetic effects that may occur when foreign chemicals are introduced into the eye but at present there appears to be no satisfactory animal model for these investigations.

6.0 *IN VITRO* TESTS

In eye irritation testing *in vitro* techniques have only recently gained prominence as possible alternatives to the use of animals. Although there is little published information on *in vitro* methods specifically designed to assess eye irritation, some techniques have been developed and reported as adjuncts to standard animal tests. Methodologies under study employ isolated eyes and corneas, mammalian cell cultures, and other test systems.

An early attempt at the practical application of findings observed *in vitro* was reported by Shapiro (1956) who studied the swelling and dissolution of rabbit corneas following immersion in sodium hydroxide solutions. Swelling and destruction of the cornea was found to occur more rapidly at higher temperatures as well as at higher concentrations. *In vivo* application of alkali for varying periods showed that corneal swelling and damage increase rapidly as a factor of exposure time. Shapiro, therefore, suggested that, for alkali exposure, immediate and continued irrigation of the cornea with cold water would retard the destructive action of the alkali on the corneal stroma.

While not strictly using an *in vitro* test, J. Leighton (personal communication, 1981) is currently developing a test system using the inflammatory response of the chick chorioallantoic membrane as a measure of the eye irritation potential of a test substance. Burstein

(1980) described the use of *in vitro* methods for determining the potential ocular effects of ophthalmological materials. Test systems include whole corneal explants, which may be maintained *in vitro* for several hours; corneal perfusion, which allows corneas to function *in vitro* for up to a few hours; and cultured cells, which are isolated from corneal tissues of animals. Transmission and scanning electron microscopy and specular microscopy have been used to monitor changes in corneal cells and in both epithelial and endothelial surfaces of isolated corneas.

6.1 ISOLATED EYES AND CORNEAS

Methods for the *in vitro* incubation of the rabbit cornea, either as a part of the whole eye or as an isolated specimen, have been investigated by Mishima and Kudo (1967). Edelhauser et al. (1976) have studied the corneal swelling rate and ultrastructure in isolated rabbit and human corneas perfused *in vitro* with intraocular irrigating solutions.

As reported in a Workshop on Ocular Safety Testing sponsored by the Cosmetic, Toiletry, and Fragrance Association (CTFA, 1980), Johnson has described the apparatus and procedure for maintaining the isolated rabbit eye *in vitro*. A reasonably good correlation was observed when the *in vitro* effects of 11 chemicals on corneal thickness and anatomical characteristics in the isolated rabbit eye were compared with published results of *in vivo* eye irritation tests of the same chemicals. The correlation was best with moderate to severe irritants.

Focusing on the importance to visual acuity of the endothelial layer of the cornea, Hull (1979) described studies in which three different groups of drugs were tested for their effects on this layer in perfused rabbit corneas. A five-minute perfusion with commercially available epinephrine 1:1000 caused rapid swelling of the corneas. The endothelial cells were markedly swollen and were characterized by cytoplasmic vacuoles and loss of organelles. The toxic agent was not the epinephrine itself, but rather 0.1% sodium bisulfite, which is included as a preservative. Diluting the epinephrine five-fold to 1:5000 prevented corneal swelling even with a three-hour perfusion. Hull recommended a 1:5 dilution of the epinephrine 1:1000 agent, used for pupil dilation during cataract surgery, to prevent corneal edema and damage to the corneal endothelium.

Similarly, benzalkonium chloride, a commonly used preservative in ophthalmic preparations, produced marked swelling of the corneal endothelial surface when tested at the clinically used concentration of 0.01%. Perfusion with a $6.5 \times 10^{-4}\%$ solution of benzalkonium chloride produced a high degree of swelling in the endothelial cells and their cytoplasmic organelles. A dilution of $6.5 \times 10^{-6}\%$ was required to prevent corneal swelling and to preserve the normal endothelial mosaic pattern and cellular integrity.

The *in vitro* effects of each of two commercially available miotic compounds, 0.01% carbachol and 1.0% acetylcholine, were investigated following perfusion for 15 minutes. Corneal swelling did not result from the acetylcholine preparation, but the carbachol preparation produced a swelling rate of 53 microns/hour during the first hour after perfusion, which decreased to about 5 microns/hour for the remaining two hours. No evidence of alteration of endothelial cell ultrastructure was revealed by the microscopic observations. These results led the author to advise caution in the current intraocular use of the carbachol preparation.

An economical and efficient utilization of animal resources in developing an eye testing system was described by Carter et al. (1973). Tests were conducted with bovine eyes obtained from a commercial slaughterhouse. Exposure of the dissected corneas to detergents in physiological solution produced an increase in hydration and changes in sodium, potassium, and chloride concentrations.

Specific *in vitro* tests may also be useful in elucidating the mechanisms of ocular irritation. Green et al. (1979) used cat eye preparations to investigate a possible mechanism by which dibenz(b,f)1,4-oxazepine produces irritant effects in the cornea. The irritancy of dibenz(b,f)1,4-oxazepine and three of its methylated derivatives was measured by determining the threshold concentrations of the compounds required to produce sensory discharges in the ciliary nerve of the excised cat eye. The guinea pig blepharospasm test was also performed. Both tests yielded a similar ranking of the order of irritancy of the compounds studied. Dibenz(b,f)1,4-oxazepine was the most potent irritant and 11-methyldibenz(b,f)1,4-oxazepine was non-irritating. These results support the hypothesis that the irritant effects of dibenz(b,f)1,4-oxazepine in the cornea depend upon the compound's ability to bind to epithelial cell membranes, since the lower irritancy of the methylated derivatives correlates well with their lower affinity for the proposed binding site.

Burton et al. (1981) described an *in vitro* method using rabbit eyes for screening substances for eye irritancy. The eyes were removed immediately after death, placed in a temperature controlled chamber, and superfused with isotonic saline. Test substances reported in the literature to be severely, moderately, or non-irritating were applied to the eyes and then removed by rinsing. An exposure period of ten seconds was selected because it provided the best discrimination between irritant and non-irritant substances. Corneal thickness measurements and slit-lamp observations were used to assess the effects. Results with the test substances *in vitro* showed a generally good agreement with published observations of eye irritation in man and animals for the same substances. Deficiencies of the method were also pointed out. These included the inability of the test system to assess the potential for recovery or to evaluate possible effects on the conjunctiva.

The authors stated that live animal experiments may still be necessary before a new chemical is used by man but that they should not be carried out before the possibility of severe irritation has been excluded using an *in vitro* test.

6.2 TISSUE CULTURE

During the CTFA Workshop on Ocular Safety Testing (1980) mentioned earlier, Chan described techniques used to isolate and culture three distinct cell lines from the rabbit cornea: epithelial cells, fibroblasts, and endothelial cells. Characteristics of the epithelial cell culture showed a good correlation between the behavior of these cells *in vitro* and *in vivo*. Chan suggests that this cell line offers the potential to evaluate toxicity by quantitating changes in the rate of cell division and migration, in the rate of terminal differentiation, and in the ability to produce multi-layer formations in culture.

The effects of a variety of antiglaucoma drugs were studied in cultures of corneal epithelium derived from rabbits and freshly enucleated human eyes (Krejci and Harrison, 1970). Test solutions were diluted 1000-fold because preliminary tests showed that higher concentrations (1:10, 1:100) caused rapid cell death in corneal epithelium in tissue culture. All control and treated corneal specimens were observed for growth of epithelial sheets and development of degenerative changes. The results were considered to be potentially beneficial in choosing the least toxic drugs for topical application to glaucomatous eyes complicated by abnormal corneal epithelium.

The suitability of other cell types has been investigated in correlations with primary eye irritation test results. The findings of Bell et al. (1979) in studies of human buccal mucosa cells exposed to a test material for 10-15 minutes closely resemble results obtained in the rabbit eye. The *in vitro* studies utilized phase contrast microscopy to determine the proportion of treated cells in which nuclei were no longer clearly visible. The authors note that these *in vitro* tests did not encompass the full range of diverse factors which influence eye irritation, including the ability of a test agent to penetrate the cornea.

A recent attempt to devise a simple, straightforward *in vitro* method as an alternative to the Draize test has been reported by Simons (1980). Since a severe irritant acts directly on cells *in vivo*, cytotoxicity is determined using the *in vitro* technique. Three shampoos that were established by the Draize test as being of high, moderate, or low irritancy were tested on an established line of mouse embryo cells chosen for its sensitivity. After the cells were cultivated in a growth medium for 18 hours, each shampoo, diluted in growth medium, was added at various concentrations to the cell-medium suspension and incubated for another 48 hours. The cells were then collected and the total number of viable cells was counted. Statistical analyses of the data showed that, although the test system differentiated the most irritating shampoo from the least irritating one, it could not differentiate the moderately irritating shampoo from the least irritating one. These results do suggest a basis for an *in vitro* system for the screening of severe irritants as an alternative to the Draize test. While the discriminatory power of the test needs improvement, the development of the technique may be a significant step in the search for a reliable *in vitro* method.

An *in vitro* assay which can be directly applied to tests of the irritancy of surfactant chemicals has been described by McCormack (U.S. Dept. of Health and Human Services, 1981). The surfactants induce the release of radioisotopically labelled serotonin into the medium of cultured peritoneal mast cells from the rat. While not all chemical compounds or classes of compounds lend themselves equally well to this test, the results with surfactants corresponded closely with those obtained by the Draize test.

The above test systems are summarized in Table 6.

Table 6. Summary of Tissue Culture Tests for Assessing Eye Irritation

Cell Type	Measurement of Irritation	Comparison with Results in Rabbit Eye Tests	Reference
Rabbit Cornea Epithelial Cells	Changes in the rate of cell division and migration, the rate of terminal differentiation, and the ability to produce multilayer formations in culture	Similar	Chan (CTFA, 1980)
Rabbit and Human Cornea Epithelial Cells	Development of degenerative changes, i.e., changes in growth of epithelial sheets	Not specified	Krejci and Harrison (1970) Krejci (1976)
Human Buccal Mucosa Cells	Proportion of treated cells in which nuclei are no longer clearly visible	Similar	Bell et al. (1979)
Mouse Embryo Cells	Cytotoxicity	Similar (discriminatory power not perfected)	Simons (1980)
Rat Peritoneal Mast Cells	Release of radioisotopically labelled serotonin into the medium	Similar (with surfactants)	McCormack (U.S. Dept. of Health and Human Services, 1981)

7.0 EXTRAPOLATION OF ANIMAL DATA TO MAN

A comparison of animal and human response in eye irritation tests is presented in Table 7. These studies include varying numbers of chemicals and, therefore, are not all of equal significance. It is evident, however, that the rabbit eye is at least as sensitive or more sensitive than the human eye in a large majority of studies on substances in which data from both species exist. This emphasizes the generally conservative nature of rabbit eye test results when applied to human risk assessment. Comparative results from other studies also show that the responses of the monkey and dog eye usually correlate more closely with the degree of response seen in humans.

Table 7. Comparison of Animal and Human Responses

Irritation Type	Test Agent	Test Species	Sensitivity of Animal Response Compared to Man	Reference
Primary Irritation	Shampoo	Rabbit *(a)*	More sensitive	Rieger and Battista (1964)
	Detergent containing neutralizer	Rabbit	Less sensitive	Rieger and Battista (1964)
	NS *(b)*	Rabbit	Less sensitive	Rieger and Battista (1964)
	Anionic surfactant formulation	Rabbit (direct instillation)	More sensitive	Buehler and Newman (1964)
	Anionic surfactant formulation	Rabbit (applicator instillation)	Similar	Buehler and Newman (1964)
	Anionic surfactant formulation	Monkey	Similar	Buehler and Newman (1964)
	Soap solution	Rabbit *(a)*	Less sensitive	Gaunt and Harper (1964)
	Sap from *Euphorbia*	Rabbit *(a)*	Variable	Crowder and Sexton (1964)

Table 7. Comparison of Animal and Human Responses (Continued)

Irritation Type	Test Agent	Test Species	Sensitivity of Animal Response Compared to Man	Reference
Primary Irritation (Continued)	Sap from *Euphorbia*	Dog *(a)*	Similar	Crowder and Sexton (1964)
	Liquid detergent	Rabbit	More sensitive	Beckley (1965a)
	Liquid detergent	Dog	More sensitive	Beckley (1965a)
	Shampoos	Rabbit *(a)*	More sensitive	Bonfield and Scala (1965)
	Various materials	Rabbit *(a)*	More sensitive	Carter and Griffith (1965)
	Various materials	Monkey *(a)*	Similar	Carter and Griffith (1965)
	NS	Dog *(a)*	Similar	Carter and Griffith (1965)
	Cytarabine hydrochloride	Rabbit	Similar	Elliot and Schut (1965)
	Cytarabine hydrochloride	Monkey	Similar	Elliot and Schut (1965)
	Detergents and phosphates	Monkey	Similar	Coulston and Serrone (1969)
	5% soap solution	Rabbit	Less sensitive	Beckley et al. (1969)
	5% soap solution	Monkey	Similar	Beckley et al. (1969)
	Detergent	Rabbit	More sensitive	Beckley et al. (1969)
	Detergent	Monkey	Similar	Beckley et al. (1969)
	NS	Rabbit	More sensitive	Giovacchini (1972)

Table 7. Comparison of Animal and Human Responses (Continued)

Irritation Type	Test Agent	Test Species	Sensitivity of Animal Response Compared to Man	Reference
Primary Irritation (Continued)	NS	Monkey	Similar	Giovacchini (1972)
	NS	Dog	Similar	Giovacchini (1972)
	Hairdressing	Rabbit *(a)*	Less sensitive	Van Abbe (1973)
	Hairdressing	Monkey *(a)*	Less sensitive	Van Abbe (1973)
	Hairdressing rinsings	Rabbit	More sensitive	Van Abbe (1973)
	Aqueous solution of hairdressing ingredient	Rabbit	Similar	Van Abbe (1973)
	Various materials:			
	0.1 ml instilled	Rabbit *(a)*	More sensitive	Griffith et al. (1980)
	0.01 ml instilled	Rabbit *(a)*	Similar	Griffith et al. (1980)
Sensory Irritation				
Blepharospasm Response	ortho-Chlorobenzylidene	Rabbit	Less sensitive	Ballantyne and Swanston (1973)
	Malononitrile	Guinea pig	Less sensitive	Ballantyne and Swanston (1973)
	Dibenzoxazepine	Rabbit	Less sensitive	Ballantyne and Swanston (1974)
	Dibenzoxazepine	Guinea pig	Less sensitive	Ballantyne and Swanston (1974)
Mouse Writhing Test	Shampoo	Mouse	Similar	Shanahan and Ward (1975)
Mouse Upper Respiratory Tract Test	Various materials	Mouse	Similar	Alarie (1966)

Table 7. Comparison of Animal and Human Responses (Continued)

Irritation Type	Test Agent	Test Species	Sensitivity of Animal Response Compared to Man	Reference
Corneal Anesthesia Induced by Test Agents	Soaps, shampoos	Rabbit	More sensitive	Harris, et al. (1975)
	Commercial shampoo formulations	Rabbit	More sensitive	Giovacchini (1972)
	Commercial shampoo formulations	Monkey	Similar	Giovacchini (1972)

(a) Study in humans not conducted. Comparison obtained from available human experience by the authors cited.

(b) Not specified

Other studies reported in Table 7 show that the instillation of volumes smaller than 0.1 milliliter into the conjunctival sac of the rabbit eye produces results of greater predictive value. Testing substances under the conditions of intended human use may also permit a more accurate indication of the human response. This was illustrated by the previously mentioned work of Van Abbe (1973) in which initial conventional testing of a commercial hair dressing in rabbits failed to detect subsequent effects reported by humans. Similarly, the value of approximating human use conditions in the testing of over-the-counter ophthalmological preparations is recognized by the Food and Drug Administration (Federal Register, 1980). The proposed test procedure for determining the safety of these materials is a modified Draize test which permits the sample to be instilled in the manner and frequency of intended use.

Experimental methods for determining the adverse ocular effects of substances are primarily valuable as comparative tools. No single species or experimental procedure is universally predictive of the human response. When available, human studies, either controlled product use trials or industrial exposure surveys, are especially valuable since they avoid the uncertainties inherent in the extrapolation of animal data to man.

8.0 APPROACHES FOR THE FUTURE

The use of intact animals for eye irritation testing is presently the only valid method for determining ocular safety. At the same time, research is underway on alternative test systems. The objective is to develop methods that are predictive of the response of the human eye to irritants, are practical and cost effective and reduce the current dependence on *in vivo* testing. Since testing in laboratory animals appears necessary for the foreseeable future, humane considerations require that methodologies at least minimize both the number of animals needed in the assessment process and the possibility of causing undue pain or discomfort. The investigation of topical anesthetic drugs is representative of the concern to reduce potential discomfort of the test animals.

A scheme similar to the following might be considered in the future to determine the eye irritation potential of a substance (see Figure 1). Initially determine the pH of the test material. Substances of pH 11.5 or greater need not be tested further since it can be assumed that they would be damaging to the eye. The same exclusion will apply to highly acidic substances of pH approximately 2 or less, although this pH limit is less well-defined than that at the alkaline end of the scale. Additional testing of selected acids in the pH 1-3 range should help to establish this lower limit more precisely.

Materials that are neither highly alkaline nor highly acidic are then tested for dermal irritation. A substance that proves to be severely irritating or corrosive to the skin would probably be severely irritating or damaging to the eye. Testing in the eye, therefore, would not be necessary. If the substance proves to be only slightly to moderately irritating to the skin, further testing should be considered.

In vitro methods of testing for eye irritation potential will continue to be developed. When fully validated, these tests may provide valuable alternative or supplementary methodologies of testing materials for ocular safety. Approaches involving tissue cultures of corneal epithelial cells or more common cell lines appear most promising. The major barrier to be overcome is the limited correlation which has been seen to date between *in vivo* effects and the results of *in vitro* tests. In addition, a primary criticism to the use of *in vitro* tests for eye irritation is their inability to measure the whole body reaction, e.g., the reversibility of lesions, delayed responses, and effects on specific ocular structures.

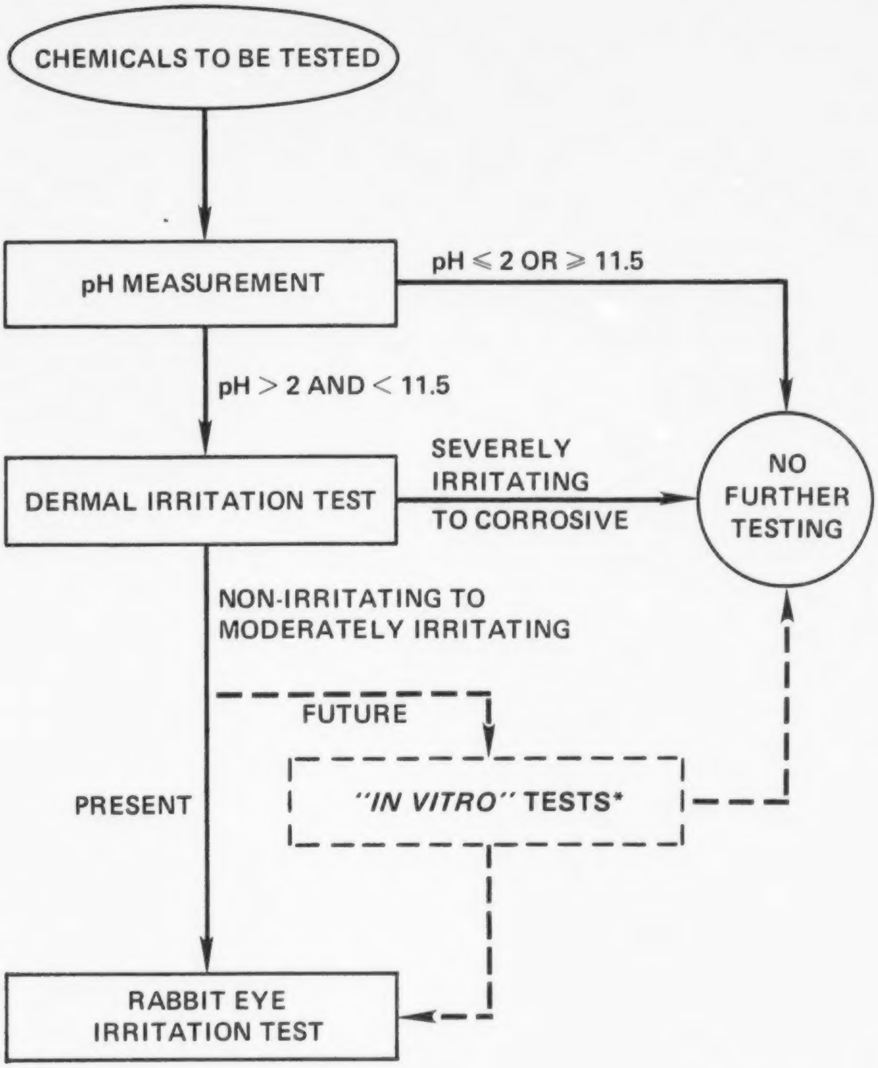
Further research is necessary to determine which responses *in vitro* will form the basis for the exclusion from testing or a requirement for testing in the intact animal. The critical measurement will be that which detects compounds that have the capability to produce ocular damage. Those scoring as severe irritants may not need to be further tested or, if exposure to the human eye is probable, the substances may more effectively be tested in the rabbit using smaller volumes or dilutions. Similarly, for substances scoring less severely, the use of *in vitro* results for test exclusions or for selecting conditions of the intact animal test may depend on anticipated product use and exposure hazards. Currently, *in vitro* tests are not adequately validated for use in the risk assessment process.

Substances which are not screened out on the basis of pH, dermal irritation test results, or *in vitro* test results would be tested *in vivo* for eye irritation. These substances typically would be expected to cause reactions ranging from non-irritating to, at the most, moderately irritating.

This tier approach for eye irritation testing would not necessarily simplify the overall evaluation process. A battery of tests as depicted in Figure 1, however, which are straightforward, inexpensive, and quick to perform, could be an extremely valuable tool if properly validated. Use of a tier approach could significantly improve the eye irritation risk assessment process with no loss in precision, predictability, or accuracy of the data and could enhance the acceptability of the methods used in the process.

9.0 CONCLUSIONS

Establishing the degree of risk associated with exposure of the eye to a substance requires the design and execution of well-planned tests. Factors important in the selection of the test method and the specific procedures to be employed include (1) economic and practical limitations, (2) the ability of the test to predict human responses, and (3) humane concerns in the use of test animals. The use of any animal species in this process involves a delicate balancing of pragmatic considerations with the requirements for reliable test results. The specific details of the test methods, as we have seen, can be tailored to the information needs of the investigator and/or the regulating authority. These details include test population size, number of doses, number of examination periods, and the addition of separate groups to receive eye irrigation. Factors such as the intended use of a new product or ingredient and the conditions under which it will be produced and distributed may also influence the selection of a particular test method.


CHEMICALS TO BE TESTED
pH MEASUREMENT
pH ≤ 2 OR ≥ 11.5
pH > 2 AND < 11.5
DERMAL IRRITATION TEST
SEVERELY IRRITATING TO CORROSIVE
NO FURTHER TESTING
NON-IRRITATING TO MODERATELY IRRITATING
FUTURE
PRESENT
"IN VITRO" TESTS*
RABBIT EYE IRRITATION TEST

**In vitro* tests will require further research and validation to determine their overall usefulness in the risk assessment process.

Figure 1. Tier Approach for Eye Irritation Testing

The guidelines recommended by the Organisation for Economic Cooperation and Development and the Interagency Regulatory Liaison Group will contribute to standardization in eye irritation testing and thereby should improve the consistency and quality of the data generated for federal regulatory purposes. An evaluation of these guidelines in relation to the available literature indicates, in general, a solid base of support for the recommended procedures.

SPECIES

In spite of several concerns, the rabbit remains the species of choice for eye irritation studies. Both the OECD and IRLG guidelines recommend that testing be performed using healthy adult albino rabbits. The literature, which consists mostly of rabbit data, also generally supports the primary use of the rabbit in eye irritation testing as a sensitive test species that provides a conservative extrapolation to man. The monkey eye is less sensitive than the rabbit eye to most substances and apparently is a more accurate predictor of human eye irritation. Cost, handling, and availability, however, restrict testing in the monkey. Other species have also been examined. These include the guinea pig, rat, mouse, hamster, chicken, dog, and cat. The use of the rat in eye irritation testing has not been adequately studied.

PREPARATIONS

Exclusion of substances from testing in the rabbit eye, based on pH or the results of dermal irritation tests, appears to be warranted. In fact, the determination of the pH and/or dermal irritation potential of a test substance normally should be performed before eye irritation testing is conducted. As to the actual pH ranges which qualify for exclusion, there is a strong basis at the alkaline end of the pH scale for assuming that substances of pH 11.5 or greater will be severely irritating or corrosive. At the acid end of the scale, although the current guideline exclusion of substances of pH 2 or less is certainly in the right range, the evidence is less clearcut and further investigation seems indicated.

Substances which score as severe skin irritants or corrosive agents in standard dermal irritation tests generally can be assumed to be at least severely irritating to the eye and need not be tested, as recommended in the guidelines. Review of a large number of test results suggests that this relationship will be valid for roughly 95% of a wide variety of chemicals. The classification of a substance as an eye irritant based on dermal irritation test results could be confirmed if questionable by performing the eye irritation test.

PROCEDURES

Initial testing for eye irritation with three animals normally will be sufficient to identify substances that are non-irritating or maximally irritating. Testing with additional animals will be necessary to fully characterize substances of intermediate degrees of irritancy (OECD) or to distinguish a positive from a negative test result for substances producing equivocal results in the initial three animals (IRLG). The conservative extrapolation in risk assessment provided by the generally high sensitivity of the rabbit eye and by the procedure of instilling a relatively large volume of material permits the use of only three animals in the initial test.

The volume of material recommended for testing by both the OECD and the IRLG is 0.1 milliliter (or 100 milligrams). This recommendation in large part is a reflection of the historical use of this volume from the time of Draize to the present. The continued use of this dose for comparative purposes seems justified. A recent study indicates, however, that the response in the rabbit eye to smaller volumes more closely approximates the response encountered in man from typical accidental exposure. Although not specifically addressed by the OECD and IRLG guidelines, the issue of multiple doses and smaller volumes has been raised by the National Academy of Sciences. The NAS suggests that the use of two or more different doses would generate more information and permit the determination of dose response characteristics of a test material. The use of smaller volumes (or greater dilution) of the test agent also is reported to permit better discrimination among substances of similar irritancy. Thus, in addition to the standardized volume, the use of smaller test volumes (or dilutions) should be considered if fuller characterization of the irritant properties of the test material is desired.

Similarly, direct instillation of the test material into the conjunctival sac has been established historically as the standard method of exposure in eye irritation tests. While direct application to the cornea probably more closely simulates accidental exposure in humans, this technique has been used only occasionally and requires further evaluation and standardization. Instillation into the conjunctival sac as recommended in the guidelines appears to be the most practical and reliable method at present. With proper control and validation, direct corneal application could be considered as an optional technique on a case by case basis.

The method of application of pressurized aerosol products apparently has not been extensively investigated. Direct timed application of a pressurized spray to the eye at a specific distance (as recommended in both the OECD and IRLG guidelines) has evolved as the preferred method for assessing the hazard posed by such products.

The IRLG guidelines do not provide specifically for separate test groups to assess the effect of irrigation with water following instillation of the test material. An optional provision does allow for washing the eyes of the test animals after the 24-hour examination, if desired. The generally beneficial effects of washing the eyes with water following exposure to an irritant have been demonstrated in a number of studies. The timing of the wash following exposure (i.e., the duration of exposure) also appears to be critical in preventing or ameliorating the irritant response. For substances found to be irritants, the use of a separate group (or groups) to accurately assess the role of irrigation in modifying the irritant response appears to be a useful adjunct. This option is provided for in the OECD guidelines. Test results on the effects of washing also can be used to evaluate the appropriateness of standard emergency procedures in the event of accidental human eye exposure to specific substances.

OBSERVATION

The recommended observation periods of up to 72 hours following instillation specified in the OECD and IRLG guidelines are adequate to define the irritation capacity of a test material. The value of extending the period of observation to determine the progress and reversibility of corneal involvement and/or the irritation reaction is emphasized by the OECD guidelines. In both sets of guidelines, however, the use of observation periods in excess of 72 hours is left to the discretion of the investigator. The general philosophy is one of flexibility sufficient to fully evaluate the hazards posed by test substances. As stated in the OECD guidelines, "The duration of the observation period should not be fixed rigidly but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed."

Aids such as fluorescein dye and various optical instruments can be used to facilitate examination of ocular reactions. The guidelines do provide for optional use of these aids, which can be advantageous in identifying and characterizing subtle changes and differences in response. The IRLG guidelines also recommend that both eyes of the test animals be examined using optical instruments, fluorescein, ultraviolet light, or other appropriate means to assess any existing abnormalities prior to testing.

EVALUATION

Given the empirical nature of eye irritation testing, the achievement of greater consistency and reliability in the scoring of lesions has long been a major goal. Modifications of the original Draize test, therefore, have included significant changes in the scoring system. Besides the original system proposed by Draize, a scoring system that is now widely used is a modified version adopted by the Food and Drug Administration in the Federal Hazardous Substances Act of 1964 and published shortly thereafter in the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances." This system eliminates the use of the multiplication factors in the original Draize system which were designed to attribute more significance to the portions of the anterior eye considered most critical to vision. This modified Draize system is recommended for use by both the OECD and IRLG guidelines and provides an adequate basis for characterizing ocular lesions and irritation. Development of the illustrated guide by the FDA contributed significantly to the reliability of the scoring process. This guide was revised and expanded by the CPSC in 1976. The method and plates from the guide are incorporated as Appendix B in the report as a reference and aid in the evaluation of eye irritation results. The OECD recommends the use of such a guide to promote standardization and uniform interpretation and reporting of the data.

Both the OECD and IRLG provide narrative descriptions of the range of irritation responses that correspond to individual numerical scores, but neither assigns descriptive terms (e.g., slightly irritating, moderately irritating) to the overall scores. In the IRLG method, the scores are used to differentiate an irritant from a non-irritant. The IRLG, however, does note that the three highest scores for corneal opacity are indicative of corrosive effects when opacities persist to 21 days. As pointed out by the OECD guidelines, individual scores should be viewed as reference values and not as absolute scores. The final classification of the irritant capacity of a substance should be based on a full description and collective evaluation of all aspects of the responses of the test animals.

APPROACHES FOR THE FUTURE

The use of intact animals for eye irritation testing is currently the only valid method for reliably determining ocular safety. The appropriateness of the OECD and IRLG guidelines for these tests has been generally confirmed in this review. Alternative methods are being developed, however, to address practical, economic, and animal welfare concerns regarding the testing process. The available data suggest that a tier approach may be feasible. Substances would be initially screened for pH, dermal irritation, and possibly for activity in *in vitro* tests when adequately validated. These preliminary tests should identify most severe irritants and significantly reduce the need for testing these substances in the eyes of intact animals.

10. RECOMMENDATIONS FOR FURTHER RESEARCH

In the course of the development of this report, a number of issues were identified which could not be resolved due to lack of adequate information. Several of these areas which could benefit from further developmental research are listed as follows:

- The pH at which highly acidic agents can be considered severely irritating or corrosive has not been established as clearly as that for highly alkaline materials. The recommendation that substances of pH 2 or less need not be tested due to their potential irritant properties needs further validation.
- The continued use of a single volume (dose) for eye irritation testing is based primarily on historical precedent. Insertion of a provision in the OECD/IRLG guidelines for the use of smaller volumes or dilutions in addition to the recognized standard dose appears to merit consideration. Additional research is warranted to further correlate findings on the degree of response of the rabbit eye to various test volumes with human eye response to accidental exposure.
- The eye irritation response produced by a test substance depends in part on the method of its application. Additional studies should be conducted to characterize the differences which can occur when a substance is instilled into the conjunctival sac as opposed to direct application to the cornea with and without applicator aids.
- Little information exists regarding the general suitability of rodent species in eye irritation testing. Further research on species such as the guinea pig and rat is clearly justified to determine if there are acceptable and practical alternatives to the use of the rabbit and to provide added flexibility in test procedures.
- Use of topical ocular anesthetics in the test process would increase the acceptability of eye irritation testing in animals. Further work is necessary to establish the ability of these drugs to reduce pain without significantly influencing the test results.
- Continuing research is needed to develop and validate a battery of adequately sensitive *in vitro* tests.
- Additional research on the testing of aerosols might be directed toward a correlation of effects with those produced by standard conjunctival sac instillation of the collected liquid. An understanding of the influence of spray pattern and aerosol characteristics would also be useful in interpreting results.

11. REFERENCES

Alarie, Y. (1966) Irritating properties of airborne materials to the upper respiratory tract. *Arch. Environ. Health* 13:433-449.

Aronson, S.B. (1975) The role of ocular irritation in evaluation of dermatotoxicity. In: Maibach, H. (ed.), Animal Models in Dermatology. Churchill Livingston, London and New York. 23-35.

Ashford, J.J. and Lamble, J.W. (1974) A detailed assessment procedure of anti-inflammatory effects of drugs on experimental immunogenic uveitis in rabbits. *Invest. Ophthalmol.* 13:(6)414-421.

Baldwin, H.A.; McDonald, T.O.; Beasley, C.H. (1973) Slit-lamp examination of experimental animal eyes. II. Grading scales and photographic evaluation of induced pathological conditions. *J. Soc. Cosmet. Chem.* 24:181-195.

Ballantyne, B. and Swanston, D.W. (1973) The irritant potential of dilute solutions of ortho-chlorobenzylidene malononitrile (CS) on the eye and tongue. *Acta. Pharmacol. et Toxicol.* 32:266-277.

Ballantyne, B. and Swanston, D.W. (1974) The irritant effects of dilute solutions of dibenzoxazepine (CR) on the eye and tongue. *Acta. Pharmocol. et Toxicol.* 35:412-423.

Ballantyne, B. and Swanston, D.W. (1977) The scope and limitations of acute eye irritation tests. In: *Current Approaches in Toxicology.* Porton Down/Wilts., Engl., Med. Div., Chem. Res. Establ. 139-157.

Ballantyne, B.; Gazzard, M.F.; Swanston, D.W. (1972) Effects of solvents and irritants on intraocular tension in the rabbit. *J. Physiol.* 226:12P-14P.

Battista, S.P. and McSweeney, E.S. (1965) Approaches to a quantitative method for testing eye irritation. *J. Soc. Cosmet. Chemists.* 16:119-131.

Bayard, S. and Hehir, R.M. (1976) Evaluation of proposed changes in the modified Draize rabbit irritation test. *Toxicol. Appl. Pharmacol.* 37(1):186.

Beckley, J.H. (1965a) Comparative eye testing: man vs. animal. *Toxicol. Appl. Pharmacol.* 7:93-101.

Beckley, J.H. (1965b) Critique of the Draize eye test. *Am. Perfume. Cosmet.* 80:51-54.

Beckley, J.H.; Russell, T.J.; Rubin, L.F. (1969) Use of the rhesus monkey for predicting human response to eye irritants. *Toxicol. Appl. Pharmacol.* 15:1-9.

Bell, M.; Holmes, P.M.; Nisbet, T.M.; Uttley, M.; Van Abbe, N.J. (1979) Evaluating the potential eye irritancy of shampoos. *Int. J. Cosmet. Sci.* 1(2):123-131.

Benke, G.M.; Brown, N.M.; Walsh; M.J.; Drotman, R.B. (1977) Safety testing of alkyl polyethoxylate nonionic surfactants. I. Acute Effects. *Fd. Cosmet. Toxicol.* 15(4):309-318.

Best, C.H. and Taylor, N.B. (1943) The Physiological Basis of Medical Practice. Baltimore, Williams and Wilkins.

Bonfield, C.T. and Scala, R.A. (1965) The paradox in testing for eye irritation. A report on thirteen shampoos. *Proc. Sci. Sect. Toilet Goods Assoc.* 43:34-43.

Buehler, E.V. (1974) Testing to predict potential ocular hazards of household chemicals. In: Winek, C.L. (ed.) Toxicology Annual 1974, Marcel Dekker. 53-69.

Buehler, E.V. and Newmann, E.A. (1964) A comparison of eye irritation in monkeys and rabbits. *Toxicol. Appl. Pharmacol.* 6:701-710.

Burstein, N.L. (1980) Corneal cytotoxicity of topically applied drugs, vehicles and preservatives. *Surv. Ophthalmol.* 25(1):15-30.

Burstein, N.L. and Klyce, S.D. (1977) Electrophysiologic and morphologic effects of ophthalmic preparations on rabbit cornea epithelium. *Invest. Ophthalmol. Visual Sci.* 16(10):899-911.

Burstein, N.L. and Maurice, D.M. (1978) Cryofixation of rabbit corneal endothelium. *Invest. Ophthalmol.* ARVO Suppl., 212.

Burton, A.B.G. (1972) A method for the objective assessment of eye irritation. *Fd. Cosmet. Toxicol.* 10:209-217.

Burton, A.B.G.; York, M.; Lawrence, R.S. (1981) The *in vitro* assessment of severe eye irritants. *Fd. Cosmet. Toxicol.* 19:471-480.

Bykov, N.F. and Semenova, D.I. (1972) The effect of local anesthetics on reparative regeneration of corneal epithelium. *Oftalmol. Zh.* 27:173-175.

Callahan, D.R.; Parish, L.C.; Nevyas, H.J. (1979) A method to evaluate the safety of an OTC ophthalmic solution in normal human volunteers. *J. Soc. Cosmet. Chem.* 30:67-71.

Carpenter, C.P. and Smyth, H.F. (1946) Chemical burns of the rabbit-cornea. *Am. J. Ophthalmol.* 29:1363-1372.

Carter, L.M.; Duncan, G.; Rennie, G.K. (1973). Effects of detergents on the ionic balance and permeability of isolated bovine cornea. *Exp. Eye Res.* 17:409-416.

Carter, R.O. and Griffith, J.F. (1965) Experimental bases for the realistic assessment of safety of topical agents. *Toxicol. Appl. Pharmacol.* 7:60-73.

Ciuchta, H.P. and Dodd, K.T. (1978) The determination of the irritancy potential of surfactants using various methods of assessment. *Drug and Chemical Toxicology* 1(3):305-324.

Cogan, D.G. and Hirsch, E.O. (1944) The cornea. VII. Permeability to weak electrolytes. *Arch. Ophthalmol.* 32(4):276-282.

Cogan, D.G.; Hirsch, E.O.; Kinsey, V.E. (1944) The cornea. VI. Permeability characteristics of the excised cornea. *Arch. Ophthalmol.* 31:408-412.

Conquet, G.D.; Laillier, J.: Plazonnet, B. (1977) Evaluation of ocular irritation in the rabbit: objective versus subjective assessment. *Toxicol. Appl. Pharmacol.* 39:129-139.

Consumer Product Safety Act. 15 U.S.C. 2051; 1972

Consumer Product Safety Commission (1976) "Illustrated Guide for Grading Eye Irritation by Hazardous Substances." Directorate for Engineering and Science. Washington, D.C.

Cosmetic, Toiletry, and Fragrance Association (1980) Workshop on Ocular Safety Testing, Washington, D.C., Oct. 7.

Coulston, F. and Serrone, D.M. (1969) The comparative approach to the role of nonhuman primates in evaluation of drug toxicity in man: a review. *Ann. New York Acad. Sci.* 162(1):681-704.

Crowder, J.I. and Sexton, R.R. (1964) Keratoconjunctivitis resulting from the sap of candelebra cactus and the pencil tree. *Arch. Ophthalmol.* 72:476-484

Davies, R.E.; Kynoch, S.R.; Liggett, M.P. (1976) Eye irritation tests - an assessment of the maximum delay time for remedial irrigation. *J. Soc. Cosmet. Chem.* 27(7):301-306.

Davson, H. (1962) The Eye. Vol. 1, Vegetative Physiology and Biochemistry. Academic Press, New York.

Dossou, K.G. and Pamart, B. (1979) Method for evaluating the eye irritation caused by shampoos and certain surface-active agents in the mouse. *Labo- Pharma- Probl. Tech.* 27(286):319-324.

Draize, J.H. (1959) Dermal Toxicity. In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States. Austin, Texas. 46-59.

Draize, J.H.; Woodard, G.; Calvery, H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Therap.* 82:377-390.

Edelhauser, H.F.; Van Horn, D.L.; Schultz, R.O.; Hyndiuk, R.A. (1976) Comparative toxicity of intraocular irrigating solutions on the corneal endothelium. *Am. J. Ophthalmol.* 81(4):473-481.

Elliot, G.A. and Schut, A.L. (1965) Studies with cytarabine HCl (CA) in normal eyes of man, monkey, and rabbit. *Amer. J. Ophthalmol.* 60:1074-1082.

Federal Register. (1964) Sept. 17. Federal Hazardous Substances Act. Title 21. C.F.R. 13009 - 191.12 Test for Eye Irritants.

Federal Register. (1972) Proposed Rule Making. April 28. 37(83):8534-8535.

Federal Register. (1978) Proposed Rules. Aug. 22. 43(163):37336-37351.

Federal Register. (1979a) Proposed Rules. July 26. 44(145):44070-44071.

Federal Register. (1979b) Aug. 15. Vol. 43, Pg. 49015

Federal Register. (1979c) Proposed Rules. Sept. 25. Vol. 44, No. 187.

Federal Register (1979d) Consumer Product Safety Commission. Code of Federal Regulations Title 16, part 1500.42, U.S. Government Printing Office, Washington, D.C.

Federal Register. (1980) Proposed Rules. May 26. 45(89):30000-30050.

Federal Register. (1981a) Interagency Regulatory Liaison Group. Jan. 22. 46(14):7075-7077

Federal Register. (1981b) Proposed Rules. Jan. 27. 46(17):8992-8993.

Floyd, E.P. and Stockinger, H.E. (1958) Toxicity studies of certain organic peroxides and hydroperoxides. *Amer. Indust. Hyg. Assoc. J.* 19:205-212.

Food and Drug Administration (1965) "Illustrated Guide for Grading Eye Irritation by Hazardous Substances." Government Printing Office, Washington, D.C.

Friedenwald, J.S.; Hughes, W.F.; Herrmann, H. (1944) Acid-base tolerance of the cornea. *Arch. Ophthalmol.* 31(4):279-283.

Friedenwald, J.S.; Hughes, W.F.; Herrmann, H. (1946) Acid burns of the eye *Arch. Ophthalmol.* 35:98-108.

Gasset, A.R.; Ishii, Y.; Kaufman, H. E.; Miller, T. (1974) Cytotoxicity of ophthalmic preservatives. *Am. J. Ophthalmol.* 78(1):98-105.

Gaunt, I.F.; Harper, K.H. (1964) The potential irritancy to the rabbit eye mucosa of certain commercially available shampoos. *J. Soc. Cosmet. Chem.* 15:209-230.

Gelatt, K.N. (1981) Ophthalmic examination and diagnostic procedures. In: Textbook of Veterinary Ophthalmology. Gelatt, K.N. (ed.), . Lea and Febiger, Philadelphia. 206-261.

Gershbein, L.L. and McDonald, J.E. (1977) Evaluation of the corneal irritancy of test shampoos and detergents in various animal species. *Fd. Cosmet. Toxicol.* 15:131-134.

Giovacchini, R.P. (1972) Old and new issues in the safety evaluation of cosmetics and toiletries. *CRC Crit. Rev. Toxicol.* 1:361-378.

Grant, W.M. (1974) Toxicology of the Eye. Thomas, Springfield, Ill.

Green, D.M.; Balfour, D.J.K.; Muir, A. (1979) Effect of methyl substitution on the irritancy of dibenz(b,f)1,4-oxazepine (CR). *Toxicology* 12(2):151-153.

Green, W.R.; Sullivan, J.B.; Hehir, R.M.; Scharpf, L.F.; Dickinson, A.W. (1978) A systematic comparison of chemically-induced eye injury in the albino rabbit and rhesus monkey. The Soap and Detergent Association, New York.

Griffith, J.F.; Nixon, G.A.: Bruce, R.D.; Reer, P.J.; Bannan, E.A. (1980) Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. *Toxicol. Appl. Pharmacol.* 55(3):501-513.

Guillot, J.P.; Caillard, L.; Gonnet, J.F.; Clement, C. (1981) Chemicals - Ocular and Cutaneous Local Tolerance-"Cosmetic," A.F.N.O.R. and O.E.C.D. Protocols. Institut Francais de Recherches et Essais Biologiques. Centre de Lyon, Les Oncins, b.p. 109, 69210 L'Arbresle.

Gundersen, T. and Liebman, S.D. (1944) Effect of local anesthetics on regeneration of corneal epithelium. *Arch. Ophthalmol.* 31:29-33.

Gupta, S. and Schiavo, D.M. (1976) A rapid testing procedure to determine low levels of ocular irritation. *Lab. Anim. Sci.* 26(3):468-472.

Harris, L.S.; Kahanowicz, Y.; Shimmyo, M. (1975) Corneal anesthesia induced by soaps and surfactants, lack of correlation in rabbits and humans. *Ophthalmologica* 170:320-325.

Harriton, L. (1981) Conversation with Henry Spira: Draize test activist. *Lab Animal* 10(1):16-22.

Heywood, R. and James, R.W. (1978) Towards objectivity in the assessment of eye irritation. *J. Soc. Cosmet. Chem.* 29:25-29.

Hood, C.I.; Gasset, A.R.; Ellison, E.D., Kaufman, H.E. (1971) The corneal reaction to selected chemical agents in the rabbit and squirrel monkey. *Amer. J. Ophthalmol.* 71:1009-1017.

Hughes, W.F. (1946) Alkali burns of the eye. I. Review of the literature and summary of present knowledge. *Arch. Ophthalmol.* 35:423-449.

Hull, D.S. (1979) Effects of epinephrine, benzalkonium chloride, and intraocular miotics on corneal endothelium. *South. Med. J.* 2(11):1380-1381.

Idson, B. (1968) Topical toxicity and testing. *J. Pharm. Sci.* 57(1):1-11.

Interagency Regulatory Liaison Group. Jan. 1981. Testing Standards and Guidelines Work Group. Recommended Guideline for Acute Eye Irritation Testing.

Jacobi, O. and Ortmann, E. (1971) Irritations of the skin and the mucosae, by shampoos. *Berufsdermatosen.* 19:(2)75-83.

Kay, J.H. and Calandra, J.C. (1962) Interpretation of eye irritation tests. *J. Soc. Cosmet. Chem.* 13:281-289.

Krejci, L. (1976) Influence of ocular drugs on the corneal epithelium. *Cesk. Oftalmol.* 32(3):163-168.

Krejci, L. and Harrison, R. (1970) Antiglaucoma drug effects on corneal epithelium. *Arch. Ophthalmol.* 84:766-769.

Krejci, L. and Krejcova, H. (1974) Changes in pupil diameter and effects on corneal tissue cultures from topically administered N-butyl gallate (COMT inhibitor) and epinephrine. *Opthal. Res.* 6:15-22.

Krueger, R. (1959) Experimentelle und klinische Beobachtungen zur Behandlung der Alkaliveratzung mit Ascorbinsaure. *Bericht. d. Opthalm. Ges.* 62:255-258.

Kuhlman, R.E. (1959) Species variation in the enzyme content of the corneal epithelium. *J. Cell. Comp. Physiol.* 53:313-326.

Laden, K. (1973) Studies on irritancy and stinging potential. *J. Soc. Cosmet. Chem.* 24:385-393.

Lallier, J.; Plazonnet, B.; Le Douarec, J.C.; (1975) Evaluation of ocular irritation in the rabbit: development of an objective method of studying eye irritation. *Proc. European Society of Toxicology.* 17:336-350.

Leuenberger, P.M. (1973) Ultrastructural changes of corneal epithelium after topical anesthesia. *Albrecht v. Graefes Arch. klin. exp. Ophthal.* 186:73-90.

MacLean, A.L. (1967) Aerosol keratitis - a common epithelial foreign-body reaction to household chemical sprays. *Amer. J. Ophthalmol.* 63(6):1709-1719.

MacLeod, I.F. (1969) Chemical Mace: ocular effects in rabbits and monkeys. *J. Forensic Sci.* 14:(1)34-47.

Marzulli, F.N. (1965) New data on eye and skin tests. *Toxicol. Appl. Pharmacol.* 7:79-85.

Marzulli, F.N. and Ruggles, D.I. (1973) Rabbit eye irritation test: collaborative study. *J. Assoc. Off. Anal. Chem.* 56(4):905-914.

Marzulli, F.N. and Simon, M.E. (1971) Eye irritation from topically applied drugs and cosmetics: preclinical studies. *Am. J. Optom.* 48:61-79.

Maul, E. and Sears, M.L. (1976) Objective evaluation of experimental ocular irritation. *Invest. Opthalmol.* 15(4):308-312.

Maurice, D.M. and Giardini, A.A. (1951) A simple optical apparatus for measuring the corneal thickness, and the average thickness of the human cornea. *Brit. J. Ophth.* 35:169-177.

McDonald, T.O. and Shadduck, J.A. (1977) Eye Irritation. In: Advances in Modern Toxicology, Marzulli, F.N. and Maibach, H.I. (eds.), Vol. 4, Hemisphere, Washington and London. 139-191.

McLaughlin, R.S. (1946) Chemical burns of the human cornea. *Amer. J. Ophthalmol.* 29(11):1355-1362.

Mel'nikova, E.A. and Rodionov, G.A. (1979) Experimental pathology of the rabbit and guinea pig eye caused by the antibiotic grisein. *Vrachebnoe Delo.* 6:85-88.

Mishima, S. and Kudo, T. (1967) *in vitro* incubation of rabbit cornea. *Invest. Ophthalmol.* 6(4):329-339.

National Academy of Sciences - National Research Council (1964) Principles and Procedures for Evaluating the Toxicity of Household Substances. Publication 1138, Washington, D.C.

National Academy of Sciences - National Research Council (1977) In: Principles and Procedures for Evaluating the Toxicity of Household Substances prepared by Committee for the Revision of NAS Publication 1138 for the Consumer Product Safety Commission. Eye Irritation. 41-54.

Olson, K.J.; Dupree, R.W.; Plomer, E.T.; Rowe, V.K. (1962) Toxicological properties of several commercially available surfactants. *J. Soc. Cosmet. Chem.* 13:469-476.

Organisation for Economic Cooperation and Development. "Acute Eye Irritation/Corrosion." OECD Publications and Information Center, Suite 1207, 1750 Pennsylvania Ave., N.W., Washington, D.C. 20006.

Pfister, R.R. and Burstein, N. (1976) The effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium: a scanning electron microscope study. *Invest. Ophthalmol.* 15(4):246-258.

Potts, A.M. and Gonasun, L.M. (1975) Toxicology of the eye. In: Toxicology - The Basic Science of Poisons. Casarett, L.J. and Doull, J. (eds.) Macmillan Publishing Co., Inc. New York, New York. 275-309.

Rieger, M.M. and Battista, G.W. (1964) Some experiences in the safety testing of cosmetics. *J. Soc. Cos. Chem.* 15:161-172.

Rowan, A.N. March 1980. The Draize Test: A critique and proposals for alternatives. Institute for the study of animal problems. Washington, D.C.

Rowan, A. (1981) The Draize test: Political and scientific issues. *Cosmetic Technology*. 3(7):32-48.

Russell, K.L. and Hoch, S.G. (1962) Product development and rabbit eye irritation. *Proc. Sci. Sect. Toilet Goods Assoc.* 37:27-32.

Sanderson, D.M. (1959) A note on glycerol formal as a solvent in toxicity testing. *J. Pharm. Pharmacol.* 11:150-156.

Seabaugh, V.M.; Bayard, S.P.; Osterberg, R.E.; Porter, W.K.; McCaulley, D.F.; Hoheisel, C.A.; Hehir, R.M.; Bierbower, G.W. (1977) Detergent toxicity survey. *American J. Public Health*. 67(4):367-369.

Seabaugh, V.M.; Osterberg, R.E.; Hoheisel, C.A.; Murphy, J.C.; Bierbower, G.W. (1976) A comparative study of rabbit ocular reactions to various exposure times to chemicals. Soc. Toxicol. 15th Annu. Meet., Atlanta, Ga., March, paper 227.

Schuck, E.A.; Stephens, E.R.; Middleton, J.T. (1966) Eye irritation response at low concentrations of irritants. *Arch. Environ. Health*. 13:570-575.

Shanahan, R.W. and Ward, C.O. (1975) An animal model for estimating the relative sting potential of shampoos. *J. Soc. Cosmet. Chem.* 26:581-592.

Shapiro, H. (1956) Swelling and dissolution of the rabbit cornea in alkali. *Am. J. Ophthalmol.* 42:292-298.

Simons, P.J. (1980) An alternative to the Draize test. In: The use of alternatives in drug research. Rowan, A.N. and Stratmann, C.J. (eds.) pp. 147-151, University Park Press, Baltimore, Md.

Smith, J.L. and Mickatavage, R.C. (1963) The ocular effects of topical digitalis. *Am. J. Ophthal.* 56:889-894.

Smyth, H.F.; Carpenter, C.P.; Weil, C.S.; Pozzani, U.C.; Striegel, J.A. (1962) Range-finding toxicity data: List VI. *Amer. Indust. Hyg. Assoc. J.* 23:95-107.

Sugar, A. (1979) Clinical specular microscopy. *Surv. Ophthalmol.* 24(1):21-32.

Sunshine, I. (ed.) (1969) Handbook of Analytical Toxicology. Sec. A, Vol. 1. The Chemical Rubber Co., Cleveland, Ohio. 585-670.

Swan, K.C. and White, N.G. (1942) Corneal permeability. I. Factors affecting penetration of drugs into the cornea. *Am. J. Ophthalmol.* 25(9):1043-1058.

Szary, A. (1977) The course of carrageenin-induced iridocyclitis in rabbits. *Arch. Immunol. Ther. Exp.* 25(6):885-890.

Trolle-Lassen, C. (1958) Investigations into the sensitivity of the human eye to hypo- and hypertonic solutions as well as solutions with unphysiological hydrogen ion concentrations. *Pharmaceutish Weekblad.* 93:148-155.

Tonjum, A.M. (1975) Effects of benzalkonium chloride upon the corneal epithelium studied with scanning electron microscopy. *Acta. Ophthalmologica.* 53:358-366.

Ulsamer, A.G.; Wright, P.L.; Osterberg, R.E. (1977) A comparison of the effects of model irritants on anesthetized and nonanesthetized rabbit eyes. *Society of Toxicology*, 16th Annual Meeting, Abs. 143.

U.S. Department of Health and Human Services (1981) "Trends in Bioassay Methodology: *In Vivo, In Vitro* and Mathematical Approaches". Initial Summary. Public Health Service, National Institutes of Health.

Van Abbe, N.J. (1973) Eye irritation: studies relating to responses in man and laboratory animals. *J. Soc. Cosmet. Chem.* 24:685-692.

Walton, R.M. and Heywood, R. (1978) Applanation tonometry in the assessment of eye irritation. *J. Soc. Cosmet. Chem.* 29(6):365-368.

Weil, C.S. and Scala, R.A. (1971) Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests. *Toxicol. Appl. Pharmacol.* 19:276-360.

Weltman, A.S.; Sharber, S.B.; Jurtshuk, T. (1965) Comparative evaluation and the influence of various factors on eye irritation scores. *Toxicol. Appl. Pharmacol.* 7:308-319.

Wright, P.L.; Ulsamer, A.G.; Osterberg, R.E. (1976) Effects of model eye irritants on corneal proteins and water content. *Society of Toxicology*, 15th Annual Meeting, Abs. 226.

APPENDIX A

Table A. Scale of Weighted Scores for Grading the Severity of Ocular Lesions (Draize et al., 1944)

I. Cornea	
A. Opacity-Degree of Density (area which is most dense is taken for reading)	
Scattered or diffuse area—details of iris clearly visible	1
Easily discernible translucent areas, details of iris clearly visible	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of Cornea Involved	
One quarter (or less) but not zero	1
Greater than one quarter—less than one half	2
Greater than one half—less than three quarters	3
Greater than three quarters—up to whole area	4
Score equals A × B × 5 Total maximum = 80	
II. Iris	
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage; gross destruction (any one or all of these)	2
Score equals A × 5 Total possible maximum = 10	
III. Conjunctivae	
A. Redness (refers to palpebral conjunctivae only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

B. Chemosis

Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

C. Discharge

Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3

Score (A + B + C) × 2 Total maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctivae.

APPENDIX B

TEST FOR EYE IRRITANTS (16 CFR 1500.42)

Six albino rabbits are used for each test substance. Animal facilities for such procedures shall be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal in the test group shall be examined before testing, and only those animals without eye defects or irritation shall be used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. For testing liquids, 0.1 milliliter is used. For solids or pastes, 100 milligrams of the test substance is used, except that for substances in flake, granule, powder, or other particulate form the amount that has a volume of 0.1 milliliter (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) shall be used whenever this volume weighs less than 100 milligrams. In such a case, the weight of the 0.1 milliliter test dose should be recorded. The eyes are not washed following instillation of test material except as noted below.

The eyes are examined and the grade of ocular reaction is recorded at 24, 48, and 72 hours. Reading of reactions is facilitated by use of a binocular loupe, hand slit-lamp, or other expert means. After the recording of observations at 24 hours, any or all eyes may be further examined after applying fluorescein. For this optional test, one drop of fluorescein sodium opthalmic solution U.S.P. or equivalent is dropped directly on the cornea. After flushing out the excess fluorescein with sodium chloride solution U.S.P. or equivalent, injured areas of the cornea appear yellow; this is best visualized in a darkened room under ultraviolet illumination. Any or all eyes may be washed with sodium chloride solution U.S.P. or equivalent after the 24-hour reading.

An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster), or inflammation of the iris (other than slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible.

The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall be regarded as negative. If two or three animals exhibit a positive reaction, the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

GRADING (CPSC, 1976)

When grading by the official method, each structure of the eye is considered independently. Damage is scored on the basis of the intensity of response of the most severely affected portion; the area of involvement is not taken into consideration (Table B). However, for purposes of illustration, the plates in this appendix are not necessarily graded on the most severely affected portion.

For completeness in judging the severity of the reaction caused by the test material, corneal lesions such as necrosis (death of tissue), pannus (subepithelial proliferation, pigmentation and accompanying vascularization of the cornea), and corneal bulging should be noted. If any of these conditions are observed during the test period, severe ocular damage is indicated.

Although not part of the official method, grading the eyes at 1 hour and at 7, 14, and 21 days after instillation of the test substance is often helpful in judging the severity of the reaction. (On Plate 6 note the 4 chemosis at 1 hour and 4 opacity at 7 days which would not have been observed during the official test period.)

As described in 16 CFR 1500.42, an optional method for reading reactions is to place one drop of fluorescein sodium ophthalmic solution (U.S.P. or equivalent) in the eye. After the excess fluorescein is flushed out with sodium chloride solution (U.S.P. or equivalent), injured areas of the cornea appear yellow. This is best visualized in a darkened room under ultraviolet illumination. Plate 3 illustrates ocular damage as evidenced by fluorescein staining.

Table B. Grades for Ocular Lesions (CPSC, 1976)

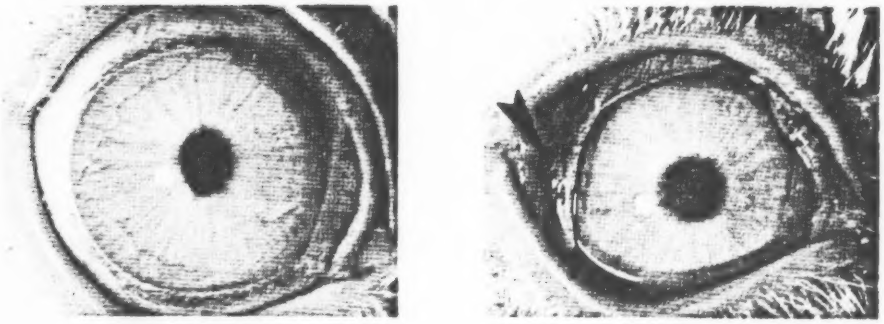
Cornea	Grade (a)
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible	4
Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these separately or combined); iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

Conjunctivae	Grade (a)
Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2*
Diffuse, beefy red	3
Chemosis	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling of lids about half closed	3
Swelling with lids more than half closed	4

(a) Asterisks indicate lowest grade considered positive.

PLATE 1

A normal eye and grades 1 through 3 redness


Normal Eye 1 Redness

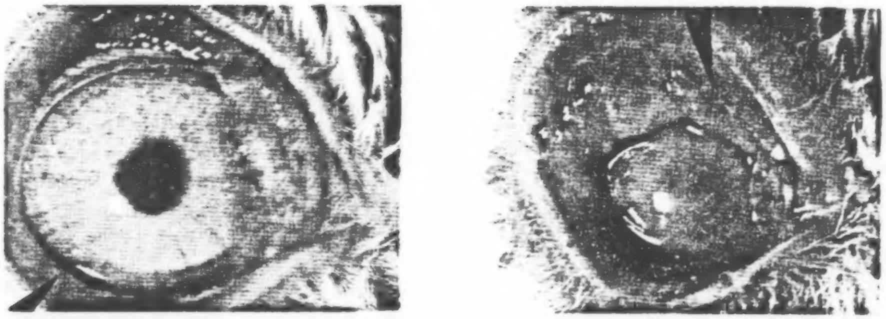
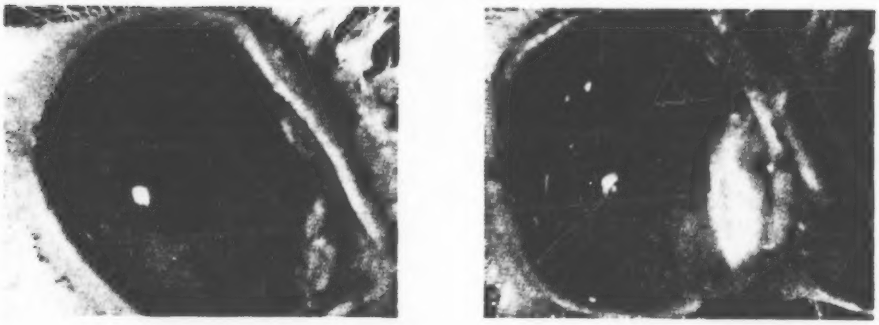

2 Redness 3 Redness

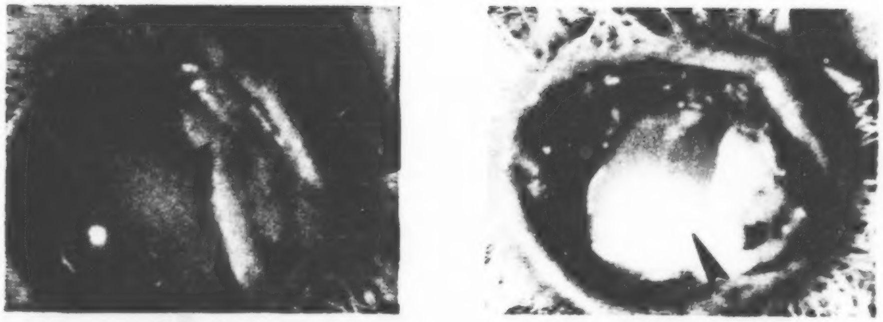
PLATE 2

Grades 1 through 4 corneal opacity



1 Opacity

2 Opacity

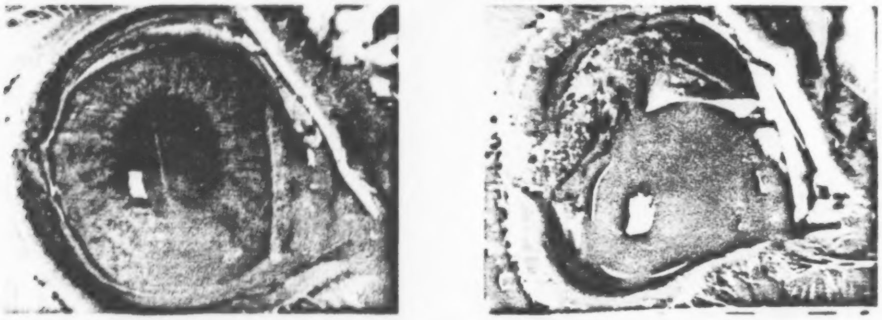


3 Opacity

4 Opacity

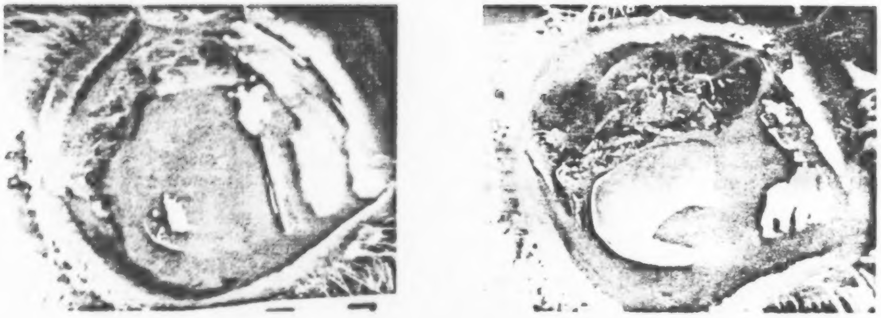
PLATE 3

The same eyes as in Plate 2 stained with fluorescein and shown under ultraviolet illumination



1 Opacity

2 Opacity

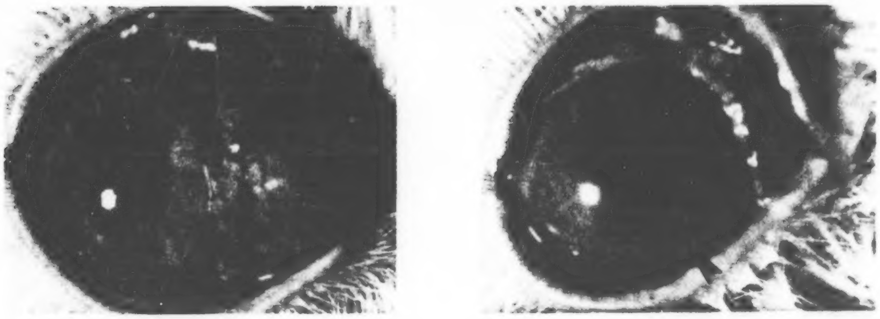


3 Opacity

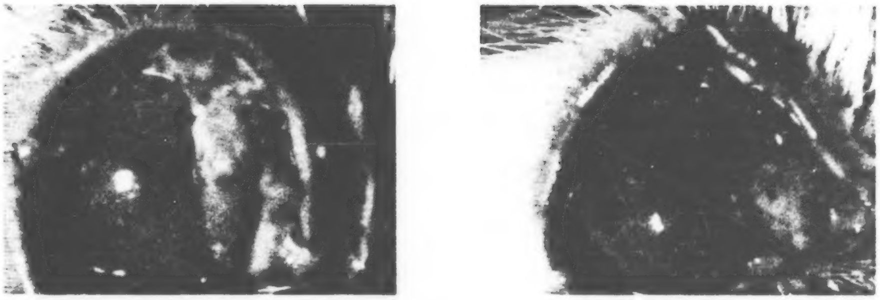
4 Opacity

PLATE 4

Grades 1 and 2 iritis

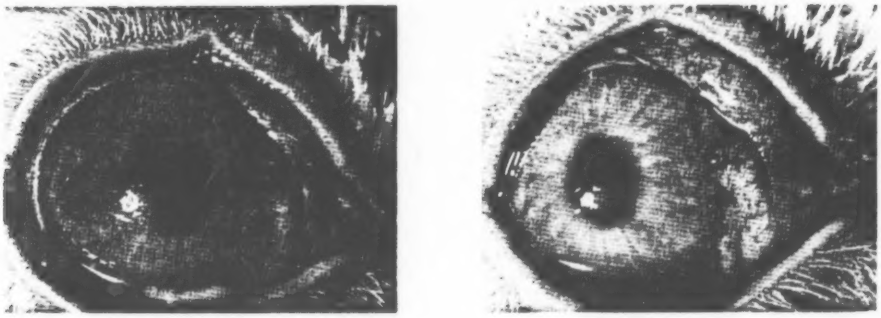


1 Iritis 1 Iritis



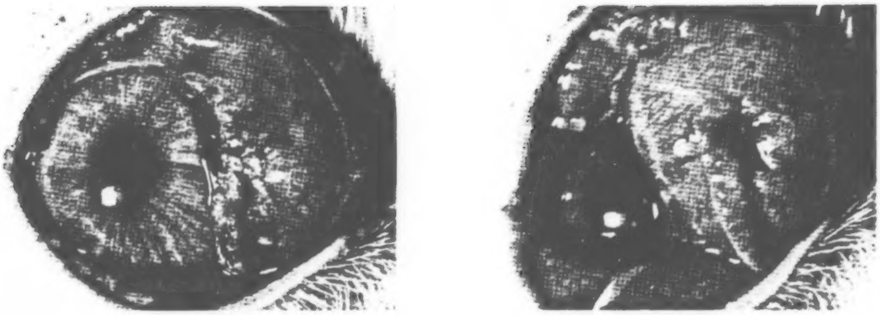
2 Iritis 2 Iritis

PLATE 5
Grades 1 through 4 chemosis



1 Chemosis

2 Chemosis



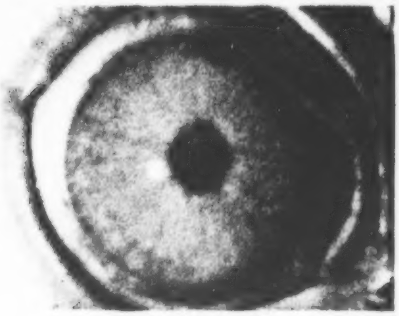
3 Chemosis

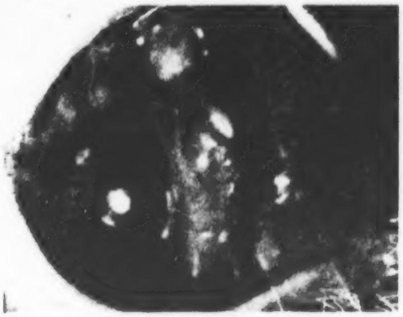
4 Chemosis

These photographs may not accurately represent chemosis because the eyes have been held open to show other aspects of irritation.

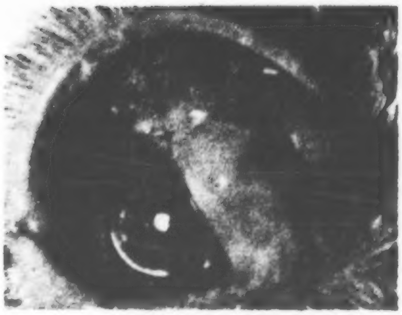
PLATE 6

The same eye prior to instillation through 1 hour, 24 hours, 48 hours, 72 hours, and 7 days after product instillation. Each eye is scored for redness, opacity, iritis, and chemosis.

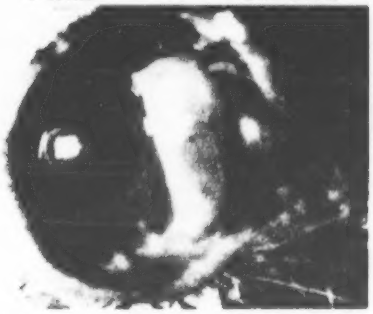

Normal Eye


1 Hour

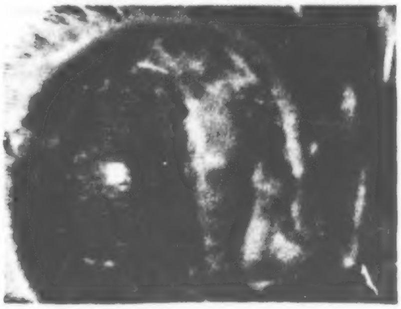
2-3 Redness >2 Opacity
1 Iritis 4 Chemosis


24 Hours

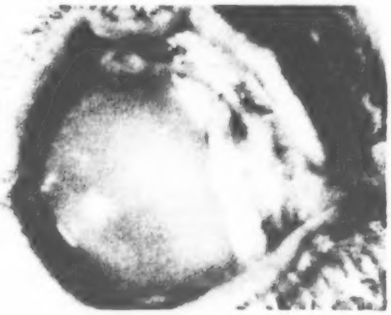
3 Redness 1 Opacity
2 Iritis >3 Chemosis


48 Hours

3 Redness >1 Opacity
2 Iritis 3 Chemosis


72 Hours

3 Redness >1 Opacity
2 Iritis >2 Chemosis


7 Days

3 Redness 4 Opacity
2 Iritis 2 Chemosis